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ON

FAT METABOLISM in MUSCULAR EXERCISE,

BY

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I N T R O D U C T I O N.

A rise in the blood fat during muscular exercise was first described in 1927 by PATTERSON (1927), whose results left no doubt as to the reality of the phenomenon. He showed definitely that there is a rise in the blood fat after prolonged muscular exercise, and that the blood fat returns to its normal level after a recovery period of about one hour. By observing the concentration of the blood corpuscles before and after exercise, he showed that the rise is real, and not merely due to concentration of the blood. PATTERSON'S suggestion that the rise in blood fat is a response to the demand of the working muscles for fat, agrees with the discovery of LAFON (1913) that the blood leaving a working muscle contains less fat than the blood entering it. The phenomenon of an alteration in the lipoid content of the blood during muscular exercise, appears to be of importance not only from the point of view of blood chemistry, but also on account of the controversy which has arisen as to whether muscle is capable of utilising fat as a source of energy. The original experimental work which will be described in/

in this thesis, was undertaken to extend the work of PATTERSON by elucidating the source and nature of the extra fat which appears in the blood, the cause of its appearance, and the relationships between the rate of utilisation of fat and its level in the blood.

In order to correlate the changes in the composition of the blood with the utilisation of fat in muscular exercise, it was found necessary to follow the complete metabolism during the period of exercise and recovery. Consequently two series of data were obtained - the one dealing with the metabolic processes going on in the whole body as observed by measurement of the respiratory exchange, and the other with changes occurring in the chemistry of the blood with particular reference to the lipoid constituents.

With the exception of PATTERSON'S work which has already been mentioned, there is no reference in the literature to work having a bearing upon the second series of data. With regard however, to the first series of data, a large amount of work has already been done, and as an introduction to our work, we propose to review briefly, the literature bearing on our problem.

We/

We shall, to begin with, consider shortly the present position of the chemistry of muscular contraction, and the performance of work by the intact animal. This will be followed by a consideration of the nature of the fuel utilised as the source of energy for muscular contraction with special reference to the utilisation of fat. Thirdly, the probable mode of utilisation of fat will be considered.

THE CHEMISTRY OF MUSCLE ACTION.

Although HERMANN (1879) discovered in 1879 that muscle can work perfectly well in the absence of oxygen, or when oxidation has been completely stopped by the addition of cyanide, it was not until 1907 that the real foundation of our present knowledge of the chemistry of muscle action was laid by FLETCHER and HOPKINS (1907) at Cambridge. These workers showed than when a muscle is made to contract anaerobically, lactic acid is produced and increases in amount up to a maximum, when lactic acid accumulation and the power of contracting stop simultaneously. If oxygen is admitted at this stage, the lactic acid slowly disappears from the fatigued muscle, until it reaches the previous resting value. Further, there is little or no accumulation of lactic acid when a muscle is made to contract in oxygen.

FLETCHER and HOPKINS concluded from these experiments that there was present in muscle a precursor of lactic acid which was transformed into lactic acid and then resynthesised from it/

it, when the muscle contracted. This process, they thought to be merely a "lubricating system", the real energy for the contraction coming from the oxidation of some other substances.

It was next shown that the lactic acid fatigue maximum produced by anaerobic contraction of an isolated muscle depends upon the state of nutrition of the muscle, and finally, MEYERHOF* (1924) was able to show that it is the accumulation of lactic acid which causes muscle to lose its power of contraction, and not exhaustion of the supply of the lactic acid precursor present in the muscle. When lactic acid is allowed to escape from muscle as it is formed, either by draining, or by buffering, a greater total amount of lactic acid is produced, and a correspondingly larger amount of work.

These discoveries led to the further discovery that the precursor of lactic acid in muscle is glycogen. The total amount of lactic acid which can be obtained from a working muscle, by neutralising it as it is formed, depends upon the stores of glycogen originally present in the muscle. MEYERHOF (1924) found that by suspending the muscle in alkaline solution buffered with phosphate, that the/

*MEYERHOF has summarised his experimental work in a review to which this reference refers.

the muscle can be made to use all its store of glycogen, and by no other method can this be accomplished.

A solution of the problem connected with the disappearance of lactic acid in the oxidative recovery of fatigued muscle was first put forward by HILL (1912-16). He made the fundamental discovery that the heat production of a working muscle is not evolved simultaneously with the contraction, but in two phases of approximately the same amount. The first phase coincides with the muscle twitch, and this he called the "initial heat". The second, he called the "delayed heat", and this is evolved during the oxidative recovery. The "delayed heat", occurs chiefly in the presence of oxygen, and HILL connected it with the oxidative disappearance of lactic acid. His first measurements were not sufficiently accurate to lead him to the correct conclusions, but MEYERHOF (1924) showed that after anaerobic stimulation, the oxygen intake is strongly increased for a definite period. This increase is closely connected with the disappearance of lactic acid, and the intake of oxygen above the resting value which MEYERHOF called the "excess oxygen", only/

only continues as long as excess lactic acid remains in the muscle. This total amount of oxygen is, however, only sufficient to oxidise one fourth to one sixth of the lactic acid which disappears. MEYERHOF (1924) showed that the remainder is converted into glycogen.

By further careful measurements of energy interchange in muscle action MEYERHOF (1924) showed how the lactic acid produced by contraction is neutralised as it is formed by the protein buffers of the muscle, and how this process accounts for a large share in the heat evolved during this phase. In the recovery phase, a corresponding amount of energy is required to recover this lactic acid preparatory to its reconversion to glycogen.

On the basis of these and his own measurements, HILL drew up a "balance sheet" which appeared to account for all the energy changes involved in muscular contraction, and which agreed with all that was then known of the chemistry of the process. Briefly, the position was that nervous stimulation of muscle caused glycogen to be converted to lactic acid, which, by its action on the muscle fibres, caused them to contract. This process could take place/

place in complete absence of oxygen, with no other energy source. When nervous stimulation was discontinued, and in the presence of oxygen, four-fifths of the lactic acid was reconverted to glycogen, the remaining fifth (or its glycogen equivalent) being oxidised in order to supply the necessary energy. This theory stresses tremendously the importance of carbohydrate in muscle action, and that it represented the complete picture, was believed until about four years ago. During the last four years, many discoveries have been made with regard to the chemistry of muscle action. These discoveries stress the extremely complex nature of the problem, a solution to which is still a long way off.

It is now known that esters of phosphoric acid play a very important part in the working of muscle. Knowledge of the precise functions of the different phosphorus compounds is still in its infancy so that it is impossible here, to give an adequate theory regarding them all. We shall confine ourselves to mentioning these compounds briefly.

The presence in muscle of hexose diphosphoric acid similar to that discovered in yeast fermentations by HARDEN and YOUNG (1906), was described by/

by EMBDEN (1914-24) and his co-workers. In 1927 however, EMBDEN and ZIMMERMANN found that the hexose phosphate occurring in muscle is the monophosphoric acid ester, and not the di-acid ester. The accepted view at the present time is that normal resting muscle contains only the monophosphoric ester. This compound can be acted upon by enzymes extractable from muscle, and converted ultimately into lactic and phosphoric acids by way of a hexose diphosphate according to the following scheme.

$$2C_6H_{11}O_5(PO_4R_2) = 2C_3H_6O_3 + C_6H_{10}O_4(PO_4R_2)_2$$

(lactic acid)

$$C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = 2C_3H_6O_3 + 2HR_2PO_4$$

In 1927 EGGLETON and EGGLETON (1927) and independently FISKE and SUBBAROW (1927) discovered a new phosphorus compound in muscle, which subsequently proved to be a compound of phosphoric acid and creatine to which the name "Phosphagen" was given. This discovery gives a reasonable explanation of the presence of creatine in muscle, and necessitates a revision of many of the views of the changes taking place in muscle during activity and recovery. Phosphagen is hydrolysed to creatine and phosphoric acid/

acid during anaerobic muscular activity, this conversion being accompanied by a large evolution of heat, and an increase in the alkalinity of the muscle. On recovery in oxygen, the phosphagen is resynthesised. MEYERHOF (1930) has stated that he now considers that muscular contraction is produced by this breakdown of phosphagen, and that the energy produced by carbo-hydrate breakdown is utilised to resynthesise phosphagen. It is also considered that phosphagen provides a readily accessible store of phosphoric acid for the esterification of hexose preparatory to its breaking down into lactic acid. The exact function of this phosphagen breakdown is not yet known, but its importance is stressed by its wide spread distribution. It is present in greatest quantity in skeletal muscle, cardiac muscle containing less, and smooth muscle a comparatively small amount. The importance of phosphagen has again been shown by recent work. LUNDSGAARD (1930) has found that skeletal muscle is able to contract even although all formation of lactic acid from glycogen or any other source, is inhibited by poisoning the muscle with iodoacetic acid. LIPMANN (1930) has shown that poisoning muscle with fluoride has the same/

same effect. The rate of decomposition of phosphagen under these circumstances is increased, and considerable esterification of hexose with phosphate and decomposition of adenyl phosphoric acid occur.

With regard to the other compounds of phosphorus occurring in muscle, little or nothing is known as to their functions. Orthophosphate occurs in resting muscle in very small amount, but in increased amount after stimulation, being produced by the hydrolysis of various phosphoric esters. Pyrophosphoric acid was discovered to be present in muscle by LOHMANN (1928). It appears to be associated with adenylic acid in equimolecular amount.

Adenylic acid is itself an ester of phosphoric acid with the purine base adenine and the pentose d-ribose. Recently, EMBDEN (1928) and PARMAS (1929) and others have studied the ammonia content of muscle, and found that there is a small but unmistakable increase as a result of injury or fatigue. It is claimed that this ammonia arises from deaminisation of adenylic acid to inosinic acid. During recovery, adenylic acid is resynthesised. The importance of this reaction in muscular contraction is not yet known, but since adenylic and

inosinic/

inosinic acids are of approximately equal strength, it might be that this reaction is part of the buffering system of muscle.

Our present knowledge of the chemistry of the action of isolated muscle, suggests that carbohydrates now play a secondary part. There is therefore no reason why carbohydrates should form the sole source of energy as was at one time thought, and there is no reason why fat should not also act as a fuel.

We shall now turn to the application of these phenomena of muscular contraction to the study of the performance of work by man. The results which have been obtained from a study of muscle contraction in isolated muscle, apply also to muscle action in the intact animal. The conditions, however, under which the contractions take place, are not the same in the two cases. Thus, for example, in the intact animal, the temperature of the working muscles is higher, they are provided with a blood supply and they are controlled from the central nervous system.

During the performance of muscular work, lactic acid appears in the blood in increased amount, showing/

showing that this substance is produced by the working muscles, whence in part it is removed by the blood stream. Lactic acid occurs in the blood of normal resting men in amounts of from 5 to 20 milligrams per 100 cc., because even when the body is at rest there is a certain amount of muscular activity which entails a constant rate of production of lactic acid. The power of the blood to remove lactic acid from muscular tissue, depends of course, upon its buffering systems. Thus MACLEOD and KNAPP (1918) have shown that when the blood is made more alkaline lactic acid is present in increased amount in the circulating blood. Also ANREP and CANNAN (1923) using the heart-lung preparation, discovered that the lactic acid content of the blood is lowered when carbon dioxide is added to it, and raised when it is removed.

The rate of accumulation of lactic acid in the blood during exercise depends upon the severity of the exercise. When the exercise is very severe, lactic acid is produced faster than it can be removed from the muscles and blood, and its amount in blood may reach a relatively high figure. When the work is only moderately severe, the oxidative processes in muscle are able to remove the lactic/

TABLE I.

	LACTIC ACID IN BLOOD mg. per cent. AT REST	AFTER EXERCISE	TYPE OF EXERCISE
MODERATE EXERCISE	20.9	36.6	Walking 3.5 miles per hour.
	21.4	58.9	Walking 4.1 miles per hour.
SEVERE EXERCISE	20.0	86.0	Standing running at 300 steps per mimute for 4 minutes, (breathing pure oxygen).
	8.5	204.0	Standing running at 239 steps per minute for 9½ minutes.

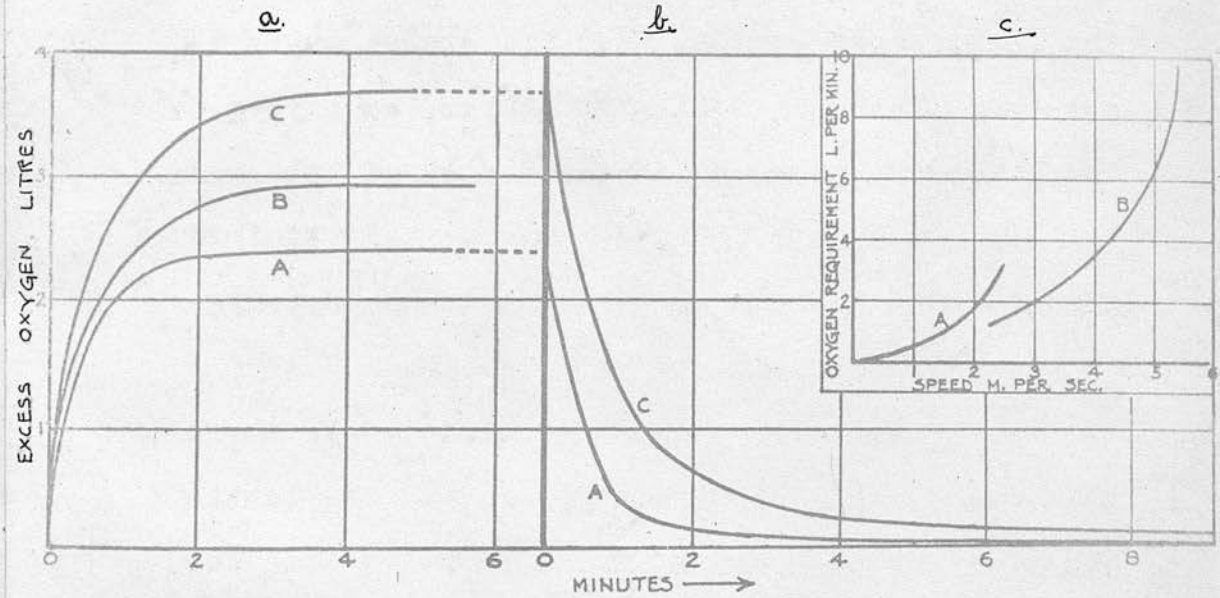
lactic acid as quickly as it is formed and the escape of acid into the blood is small. The following table, (Table I) taken from experiments by HILL, LONG and LUPTON (1924) shows the type of changes which take place in the lactic acid content of the blood with exercise of different severity. (TABLE I).

A knowledge of the oxygen requirement of exercise is of value in furnishing a measure of its severity. As a result of severe muscular exercise there is an accumulation of lactic acid in the muscles and in the blood in spite of oxidative recovery processes which go on simultaneously. There is a limit to the amount of oxygen which the body can utilise in a given time. This limit is fixed, not by the oxygen requirements of the body, but by the heart, which is unable to pump blood quickly enough to supply to the tissues the oxygen which will enable them to oxidise the lactic acid as quickly as it is formed. It is to be expected that this accumulated lactic acid will be oxidised during the period of recovery after the exercise is over. CAMPBELL, DOUGLAS and HOBSON (1919) were first to show that the oxygen intake is increased above the basal level, not only during exercise, but also for some time after the exercise/

exercise has ceased. During severe exercise the oxygen intake may remain at its maximum value, but the lactic acid continues to accumulate in muscle and in blood. In HILL'S words:- "In such a state, the muscle has to 'go into debt' for oxygen, to obtain its energy on the 'security' of a concentration of lactic acid which it will require future oxygen 'intake' to eliminate". Just as in the case of isolated muscle, the lactic acid accumulated during exercise is restored to glycogen after the exercise is completed, the energy necessary for the conversion being obtained by the combustion of some fuel such as carbohydrate or fat in the body.

When exercise of only moderate severity is performed, the oxygen intake may be able to balance the oxygen requirement. There is, in this case, only a small oxygen debt and since the concentration of lactic acid in the body does not rise very considerably above the resting value, this type of exercise may be kept up indefinitely. This balance is reached when the lactic acid content of the muscles has risen sufficiently to provoke an oxygen usage great enough to keep pace with the lactic acid production. Exercise of this type is called the "steady state", of exercise and the more severe the exercise/

FIGURE I.



I. a. Excesss Oxygen intake at commencement of work of different degrees of severity. In A and B the steady state is reached. In C the limit of supply is reached.

b. Recovery excess intake of oxygen after work. A and C represent recovery from work comparable with that in the curves A and C of a.

c. Relation between speed of locomotion and oxygen requirement. Curve A. walking; curve B. running.

exercise, the higher will be the oxygen usage and the lactic acid content when the steady state is reached. There is still, of course, an oxygen debt which depends also upon the severity of the exercise.

In Figure I. are shown curves obtained by HILL, LONG & LUPTON, (1924) showing the oxygen intake with work of varying severity.

These authors have concluded from their experiments on violent exercise, that the lactic acid which accumulates during exercise is removed during recovery in two processes. The one process is rapid, and corresponds with the oxidative removal & restoration of lactic acid in that muscular tissue in which it has formed and accumulated; the other is much slower and corresponds with the oxidation of that lactic acid which has diffused away from the working muscles into the blood stream and been carried to other parts of the body.

The production of lactic acid, and the accumulation of oxygen debt in the intact animal have been dealt with rather fully, because they have important bearings upon the employment of respiratory data to follow metabolic changes occurring in the body due to exercise and recovery. This point will be discussed later.

THE UTILISATION of FAT by MUSCLE.

ISOLATED MUSCLE.

While there is abundant evidence that carbohydrate is used as a source of energy by isolated muscle, the evidence concerning the utilisation of fat is very meagre, and not convincing. LEATHES (1906) tetanising the muscles of one side of an animal, and then comparing them with the muscles of the other side, could find no evidence for the combustion of fat on the tetanised side. WINFIELD (1915) made a similar observation, but PALAZZALO (1912) found that the fat content of stimulated muscles was less than that of unstimulated controls. That under certain circumstances, muscle can use fat coming into it, is indicated by the experiments of LAFON (1913) who observed an increase in the fat removed from blood circulating through the working muscles, compared with that removed while they were at rest. This observation is an isolated one, and has not been confirmed, so that, on the whole, the evidence on this subject is not conclusive.

With regard too, to the respiratory quotient of isolated muscle, very few observations exist. MEYERHOF (1929) found the respiratory quotient of minced frog's muscle to be in the neighborhood of unity, and this observation has been used as/

as an argument that carbohydrate is the sole source of the fuel of voluntary muscle. The evidence with intact isolated muscle, however, is not so conclusive. MEYERHOF & HIMWICH (1924) using the diaphragm of a rat, found that the respiratory quotient was not dependent upon the previous diet, and tended to be high. (av. 0.95). TAKANE (1926) also using the rat diaphragm found that the consumption of fuel by the tissue, as measured by the respiratory exchange, exceeded that which could be provided for by carbohydrate and protein and so he concluded that fat must have been oxidised. HIMWICH & CASTLE (1927) and later HIMWICH & ROSE (1929) found the respiratory quotient of isolated muscle to be the same as that of the intact animal, and to remain the same during contraction as during rest. The high experimental error (0.16) in these experiments, however, renders difficult their absolute acceptance. The only conclusion which can be drawn from the experiments on the respiratory quotient of isolated muscle is, that apart from MEYERHOF'S single observation on minced muscle, there is no evidence that only carbohydrate can be utilised. The theories of the chemistry and physiology of muscular contraction give no a priori reason for the assumption that fat cannot be utilised directly as a source of energy, although the great importance placed/

placed on the glycogen -lactic acid cycle, led many workers to believe that this was so.

THE INTACT ANIMAL.

Since the evidence for the utilisation of fat in exercise by the intact animal depends upon the value of the respiratory quotient, it is necessary first, to consider whether the lowering of the respiratory quotient during exercise and recovery may be due to increased protein metabolism. The evidence is practically unanimously against any such utilisation. PETTENKOFER & VOIT (1866) first showed that muscular work does not increase protein metabolism as measured by the nitrogen excretion, and this was confirmed by SUCCI. Later, SCHAFFER (1908) obtained the same result and so also did KOCHER (1914), The data of CATHCART & BURNETT (1926), however, suggest that up to 30% of the external work might receive its energy from protein, but CHAMBERS & MILHORST (1927) have pointed out that the exercise was in this case of short duration, and that the increased protein metabolism may only have been a temporary effect, such as the removal of 'deposit' protein.

The evidence for the utilisation of fat in muscular exercise, by the intact animal, is much more/

more abundant. The earliest experiments were performed by CHAUVEAU (1896), who found that the resting respiratory quotient rose with exercise, and formulated the theory that muscular exercise was performed at the expense of carbohydrate. Then ZUNTZ (1898) after a long series of experiments, upheld the view that fat could be oxidised in exercise. He based his conclusions partly on experiments showing that the respiratory quotient of exercise was as much a function of the previous diets as the resting respiratory quotient. This was confirmed by FRENTZEL & REACH (1901), and later by BENEDICT & CATHCART (1918), and KROGH & LINDHARD (1920). ANDERSON & LUSK (1917), showed that the respiratory quotients of a starving dog during long continued exercise on a treadmill, were practically theoretical for the combustion of fat (0.71 to 0.73), and similarly, MEYERHOF & HIMWICH (1924) obtained respiratory quotients of 0.70 for rats which had been fed on a diet containing excessive amounts of fat, during exercise on a treadmill.

Certain of these earlier experiments could be criticised in that they did not cover the full exchange of recovery as well as exercise, and the great importance of the recovery period was shown by HILL, LONG & LUPTON, (1924), In a series of researches/

researches, HILL, LONG & LUPTON, (1924) FURUSAWA (1925), and FURUSAWA, HILL, LONG & LUPTON, (1926), showed that in mild exercise of short duration, respiratory quotients in the neighbourhood of unity were always obtained, no matter what the previous diet had been. As the exercise became more violent, the respiratory quotients tended to fall. They explained these results by the conclusion that carbohydrate was the only substance oxidised, and that in prolonged or more violent exercise fat was drawn upon, and converted into sugar to supply the necessary energy, with a resultant fall in respiratory quotient.

RAPPORT & RALLI (1928) performed experiments on dogs with mild exercise (1) of short duration, and they found that the animals oxidised the same percentages of carbohydrate and fat, as when at rest, and that the muscles, like other tissues of the body, oxidised carbohydrate or fat, or both, depending upon the proportions in which those substances were presented to them in available form.

MARSH (1928), using human subjects doing moderate work, found that the respiratory quotient of exercise and recovery is a function of the previous diet, while WILSON, LEVINE, RIVKIN & BERLINER (1928), have also observed in children, a close/

close resemblance between the respiratory quotient of exercise and recovery, and that of the previous rest period. For mild exercise, this observation has recently been confirmed by BEST, FURUSAWA & RIDOUT (1929), and by RAPPORT (1929). However, with violent exercise, BEST FURUSAWA & RIDOUT obtained respiratory quotients of unity and over, values as high as 1.70 being obtained. They were unable to offer any explanation of these results, and concluded that blowing off carbon-dioxide could not account for the large volumes required to produce these high respiratory quotients. The type of exercise they employed consisted of sprinting - a very violent form of exercise and of short duration. This violent exercise produces an exceedingly high concentration of lactic acid in the tissues, a relatively large fraction of which would be excreted by the kidneys. If this lactic acid is excreted with ammonia, it is taking away ammonia which, along with carbon-dioxide, would otherwise be excreted as urea. This carbon-dioxide, together with a further amount washed out by over breathing, might conceivably account for their results.

It is to be expected, since the disease diabetes involves diminished ability to oxidise carbohydrate, that people suffering from this disease or/

or animals in which the disease has been simulated experimentally by the administration of phlorizin, would be forced to use a large proportion of fat in the performance of muscular work. GRAFE & SALOMON (1922), found that when a diabetic, with a resting respiratory quotient of 0.72 was made to exercise the resulting respiratory quotient was 0.71, RICHARDSON & LEVINE (1925) also failed to observe a rise in the respiratory quotients of exercising diabetics. In the experiments of HERTZEL & LONG (1926), the respiratory quotient of exercise in diabetes, was 0.80 compared with a resting respiratory quotient of 0.76. Also, RAPPORT & RALLI (1928,2) found that in the phlorizinised dog, there was no greater tendency for the oxidation of sugar in exercise than when at rest.

An explanation of the variations in the results obtained from the study of the respiratory exchange of exercise, is probably to be found in the great differences in the type of exercise chosen by the different experimenters. Difficulty seems to have been experienced in striking exercise of a steady average rate, the work performed being, as a rule, either too mild to produce much change in metabolic processes, or too violent to enable respiratory data to be used with proper confidence. However, the evidence from the respiratory quotient, as a whole, does not justify the belief that carbohydrate alone is oxidised to supply the fuel for muscular exercise.

MODE of UTILISATION of FAT by MUSCLE.

Although the belief appears to be justified from the evidence, that fat is used as a source of energy in muscular exercise, it remains to discuss the mode of its utilisation. There are only two possible mechanisms:; -

(1) That fat is directly burned, and

(2) That it is first converted into carbohydrate, which is then burned.

The respiratory quotient gives no information on this point, because, in either case, the end products of fat metabolism will be carbon dioxide and water.

The evidence for and against the conversion of fat to carbohydrate in the animal body hinges mainly upon two things:-

The study of the effect of various diets upon diabetic subjects and upon phlorizinised dogs, and the relative efficiency of fat and carbohydrates as sources of energy.

LUSK (1928), has recently reviewed the evidence dealing with the disease diabetes, and he concludes that this evidence is totally against any conversion of fat to carbohydrate.

In the'complete'diabetic, totally unable to oxidise glucose, the excretion of sugar is never greater/

greater than can be accounted for by the conversion of protein to carbohydrate, and in "incomplete" diabetes, it is invariably less than this. Moreover, calculation shows that the observed heat production, respiratory quotient, sugar, nitrogen and hydroxybutyric acid excretion, cannot be accounted for on the supposition that fat is converted to carbohydrate, but can be accounted for on the supposition that protein is converted to carbohydrate, while fat is oxidised in the usual way, as far as hydroxybutyric acid. Another suggestive piece of evidence leading to the same conclusion is that alteration of the protein content of the diet leads to a parallel alteration in the sugar excretion, whereas no connection whatever is observed between the fat content of the diet and the excretion of sugar.
MANDEL and LUSK, (1903) .

In considering the results of experiments upon phlorizinised animals, which show an excretion of sugar greater than can be accounted for by the protein ingested, [SOSKIN, (1929)], it must always be borne in mind that the musculature clings tenaciously to glycogen [RINGER, DUBIN and FRANKEL, (1921)], long after the other stores are completely exhausted/

exhausted, and excreted sugar unaccounted for by protein metabolism, probably comes from this source. This source too, is the origin of the increased sugar elimination by phlorizinised dogs after the administration of a single dose of adrenalin, (EPPINGER, FALTA and RUDINGER, 1908). Administration of a second injection produces no further change upon the sugar excretion RINGER (1910), WOODYAT (1913), PALMER (1917), SEUFFERT and HARTMANN (1929) .

On the basis of these and many similar facts it may be accepted that the diabetic does not convert fat to carbohydrate, and bearing in mind the circumstances of the disease, it is a legitimate conclusion that he cannot do so. This, however, affords no proof of a corresponding inability in the normal subject, although it is very strongly suggestive.

More direct evidence, in the case of normal animals, is obtained from a study of the relative efficiency of fat and carbohydrate as sources of fuel in muscular exercise. If fat is oxidised directly, there will probably be no loss of efficiency when it acts as a source of energy for muscular exercise. If, however, it is first converted into carbohydrate/

carbohydrate, the least possible wastage of energy is some 25 per cent., and probably greater than this if the reaction proceeds by a mechanism more in accordance with our knowledge of the chemistry of the two substances. Experimental evidence, were it to show conclusively that muscular work is performed with greater efficiency when carbohydrate is the source of energy than when fat is the source of energy, would suggest that fat is first converted to carbohydrate before being utilised. It would give no proof however, because it might well be that the body is less able to make use of the energy supplied directly by fat oxidation. Again, it must be pointed out that unless fat forms the sole source of energy, the total loss of efficiency is not to be expected, but some lower value, depending upon the relative amount of fat being used. Further, owing to the complete lack of knowledge concerning any mechanism which may exist in the body for the conversion of fat to carbohydrate, it is necessary to assume that all the carbon of the fat molecule is converted into carbohydrate carbon. If, as will be shown later, the conversion takes place by a mechanism which is at present known to be possible chemically, the loss of energy/

energy when fat is used as a fuel will not be 25 per cent as in the former case, but approximately 80 per cent.

CHAUVEAU (1897-98) first deduced that fat is less efficient than carbohydrate as a source of energy for muscular work, but his calculations have since been shown to be untenable. In ZUNTZ'S laboratory, HEINEMANN (1901) was unable, in human subjects, to observe any appreciable difference in their efficiencies on fat and carbohydrate diets. FRENTZEL and REACH (1901), however, found that they could perform work more efficiently on a carbohydrate than on a fat diet. The results of ATWATER and BENEDICT (1903) are somewhat discordant, sometimes showing a difference in the efficiencies, and sometimes not. Continuing these investigations, however, BENEDICT and MILNER (1907) found that, on the whole, exercise was performed about 11 per cent more efficiently on a carbohydrate than on a fat diet, but BENEDICT and CATHCART (1913) found practically no difference in the two efficiencies. ANDERSON and LUSK (1917) in experiments on a dog, found that carbohydrate is about 6 per cent more efficient as a source of energy, than fat.

KROGH/

KROGH and LINDHARD (1920) in a careful and exhaustive study of the efficiencies with which human subjects perform work on different diets, came to the conclusion that on a carbohydrate diet, exercise is performed at the expense of 11 per cent less energy than on a fat diet.

The evidence on the whole appears to warrant the conclusion that muscular work is performed somewhat more economically at the expense of carbohydrate than at the expense of fat, but further than this, it is at present impossible to go.

EXPERIMENTAL.

PREPARATION OF THE SUBJECT.

The subjects used in the experiments about to be described, with one or two exceptions noted later, though not specially trained, were all healthy young men, used to taking moderate exercise, and were on a normal mixed diet. At the time of the experiment they had fasted since the preceding evening, that is, for thirteen to fifteen hours. They had either spent the night in the laboratory, or had rested there in bed since the early morning. In either case, a state as nearly basal as possible was assured at the commencement of the experiment. For those experiments involving measurements of the gaseous exchange, this condition was obviously essential, since it provided the only possible base line from which to measure the excess metabolism during the exercise. Further, preliminary experiments showed that any previous activity on the part of the subject considerably modified the effect of a given amount of muscular work on the level/

level of the blood fat. These precautions were not observed by PATTERSON in all of his experiments, which doubtless explains some of the quantitative discrepancies between his results and these about to be described.

MODE OF EXERCISE.

In order that the exact amount of work done could be accurately calculated, the subjects were exercised on a bicycle ergometer at a steady rate. To most of the subjects cycling was an unaccustomed form of exercise, and it was found impossible for them to work for long at a greater rate than about 1,100 kilogram metres per minute. Short periods of work such as were employed by PATTERSON in his running experiments were not, therefore feasible, as amount rather than rate of work seemed to be the most important factor.

EXPERIMENTS INVOLVING MEASUREMENT OF RESPIRATORY EXCHANGE.

After the subject had reached basal conditions and had emptied his bladder, his expired air was/

was collected in a 200 litre spirometer for a period of twenty-five minutes. A sample of blood was then withdrawn and the bladder was again emptied. The work was then begun, and at intervals during its progress, the expired air was collected for a period of one minute, the subject being connected to the spirometer for this purpose without interruption of the steady rate of work.

A number of preliminary experiments satisfied us that extrapolation from the results of these separate one minute collections gave nearly as accurate a figure for the gaseous exchange over the whole period, as the much more cumbersome and uncomfortable procedure of collecting all the expired air throughout the exercise. It was, in fact, found difficult to wear the mask continuously and still work at the necessary speed over periods varying from ten to forty-five minutes. The gaseous exchange reached a steady value about five minutes from the commencement of exercise, and it was therefore necessary to confirm this in each case, by taking two samples during the latter part of the exercise with, in addition, one sample at the second or third minute. On the completion of the work, the subject immediately lay down again, and was once more connected

to/

to the spirometer. A second sample of blood was then taken. Collection of the expired air was continued until the subject had again reached an approximately basal condition, as considered by pulse and respiration rate, and minute volume of expired air. At the end of this period, usually an hour was allowed - the subject again emptied his bladder and a third sample of blood was procured.

EXPERIMENTS NOT INVOLVING MEASUREMENT OF RESPIRATORY EXCHANGE.

In two series of experiments, it was not feasible to make measurements of the respiratory exchange. The first of these was undertaken in order to determine the effect of a short period of work following immediately after apparent recovery from a first of sufficient magnitude to produce a rise in the blood fat. In one or two of these the respiratory exchange was determined over the first part of the experiment, but it was found that the discomfort of the mask worn over such a long period of time precluded the proper return to basal conditions after the second period of exercise. In these experiments/

experiments blood was withdrawn before and after each period of exercise.

The second series had as its object the determination of changes in the blood chemistry during the progress of exercise, and blood samples were withdrawn, not only before and after, but at intervals during the performance of work. This procedure necessitated interruption of the exercise, and though the withdrawal of samples was performed as expeditiously as possible, the interruption rendered fallacious any measurement of the respiratory exchange with the means at our disposal.

TABLE II.

SUBJECT.	NITROGEN EXCRETED DURING EXERCISE AND RECOVERY.	NITROGEN EXCRETED DURING SOME TIME UNDER BASAL CONDITIONS.
R.G.	1.23 grams.	1.36 grams.
D.M.D.	0.80	0.80
C.P.S.	0.79	1.03
J.B.R.	1.28	1.05
D.S.	1.29	1.25
R.E.I.	1.08	1.18
De V.	0.75	0.84
W.McL.	0.35	0.49

CHEMICAL METHODS.

The respiratory exchange was calculated in the usual way by analysis,-using HALDANE'S method - of samples removed from the spirometer.

The urine was analysed for total nitrogen in order to determine whether any of the extra metabolism during exercise and recovery was due to protein metabolism. It may be said at once that no change in the rate of nitrogen metabolism was found during the experiment. (TABLE II).

In the various blood samples were estimated total fat, soap, total cholestrol, lipoid phosphorus, the iodine number of the fatty acids, and the carbon dioxide combining power.

The method of VAN SLYKE and NEILL (1924) was used to estimate the carbon dioxide combining power of the blood.

TOTAL FAT OF BLOOD.

The method used to estimate the total fat in blood was that of STEWART and WHITE (1925) who have applied the hydrolytic method of estimation to small quantities of blood. The utilisation of this method, with any degree of accuracy, with the small amount of fat obtainable from 2 cc. of blood, was rendered possible by the introduction of the Rehberg micro-burette, which delivered 0·10 cc. of solution, and can be used for titrations requiring fractions of that amount with at least as great accuracy as is attainable with the 10 cc. of the macro-titration.

2·0 cc. of blood were added, with constant shaking to about 30 cc. of alcohol ether mixture (three parts alcohol, one part ether) in a 50 cc. standard flask. The mixture was brought just to boiling point, cooled and made up to 50 cc. with alcohol ether. After thorough mixing, it was allowed to settle and 25 cc. of the clear supernatent liquid were withdrawn for analysis. After evaporation of the alcohol ether, 5 cc. of $\frac{N}{10}$ NaOH and 5 cc. of absolute alcohol were added, and the mixture allowed to evaporate almost to dryness on the water bath, a process which occupied about 2 hours.

After/

After hydrolysis, 5 cc. of $\frac{N}{10}$ HCl were added to the mixture. This exactly neutralised the whole of the sodium hydroxide, and liberated the fatty acids which had been produced during hydrolysis. The mixture was now evaporated down almost to dryness, and the residue extracted with boiling absolute alcohol, the washings being transferred to a 10 cc. volumetric flask, and made up to volume. 1 cc. of this final solution, corresponding to one-tenth of the amount of fat originally taken, was titrated with $\frac{N}{10}$ sodium hydroxide from the Rehberg burette. The sodium hydroxide used in the titration, must be free from carbonate. In these experiments, it was kept in a paraffined bottle provided with a syphon and tap, the air inlet being guarded by a soda-lime tower. In this way the standard NaOH was prevented from coming into contact with the air. The indicator used was phenolpthalein, ·2 cc. of ·25% in alcoholic solution, and a correction was applied for the amount of indicator, volume of fluid, etc. The correction factor was found by making 10cc. of $\frac{N}{10}$ HCl up to 50 cc. with absolute alcohol in a volumetric flask, and titrating 1 cc. portions of the resulting mixture, with $\frac{N}{10}$ NaOH from the Rehberg burette/

burette using the same concentration of phenolphthalein as in the fatty acid titrations.

It must be emphasised particularly that for these estimations of blood fat, both the reagents and apparatus were accurately standardised. Thus if the hydrochloric acid be slightly below $\frac{N}{10}$, or of slightly less than 5·0 cc. be added, the fatty acid will be incompletely liberated. Conversely, if acid be added in slight excess it will remain to be neutralised in the titration. In either case, apparently slight variations may lead to serious errors, notwithstanding the subsequent dilution, since the volume of the titrating fluid is so small. All precautions were taken to ensure that the solutions used were kept in a state of purity, all being protected from the air. The alcohol used was redistilled from potassium hydroxide and the ether was re-distilled from sodium. As to apparatus, the same pipette was used to deliver the 5 cc. of NaOH, and of HCl, great care and delicacy of manipulation being observed.

This method of STEWART and WHITE has been criticised by STODDART and DRURY (1929) on the grounds of difficulty of technique, and they put forward an alternative/

TABLE III.

BLOOD SAMPLE	BLOOD FAT CONTENT	
	a.	b.
1.	619 mgs. %	609 mgs. %
2.	502 mgs. %	512 mgs. %
3.	609 mgs. %	612 mgs. %
4.(after exercise)	709 mgs. %	728 mgs. %
5.(after exercise)	899 mgs. %	911 mgs. %
6.	686 mgs. %	686 mgs. %
7.	851 mgs. %	868 mgs. %
8.(diabetic)	708 mgs. %	722 mgs. %

alternative method based on filtration of the free fatty acid after neutralisation of the added sodium hydroxide. We, however, have found no difficulty in obtaining duplicate analyses on both blood and known solutions of pure tripalmitin, agreeing to within 5 per cent. In Table III. are shown the results of some of these analyses.

Although STEWART and WHITE have shown the absence of any interfering substance such as aceto-acetic acid, and hydroxybutric acid etc., (present to an appreciable extent in pathological specimens of blood) in the alcohol-ether extract, it was realised that the phosphoric acid, produced by hydrolysis of the phospholipins of the blood, is titrated with the fatty acids.

To overcome this, a modification of the original STEWART and WHITE method, somewhat resembling the method of STODDART and DRURY was introduced. As before the fats were extracted from 2 cc. of blood by 50 cc. of alcohol-ether mixture, and 25 cc. of the resulting clear solution used for analyses. After evaporating off the alcohol-ether, 5 cc. of $\frac{N}{10}$ NaOH, and 5 cc. absolute alcohol were added and the mixture evaporated almost to dryness on the steam bath. A slight excess of $\frac{N}{10}$ HCl was now/

TABLE IV.

BLOOD SAMPLE.	FAT MGS. %		LIPOID PHOSPHORUS MGS. %	
	ORIGINAL METHOD.	MODIFIED METHOD.	OBSERVED.	CALCULATED.
1.	654,657 mgs. %	501,521 mgs. %	15.0 mgs%	16.0 mgs. %
2.	659,679	444,454	20.1	24.0
3.	426,429	331,338	12.3	10.4
4.	461,491	320,350	16.4	16.0
5.	659,660	446,442	22.5	24.0

now added, and the mixture well shaken to ensure complete separation of free fatty acids. The fatty acids were liberated in a fine emulsion, coalescence of the fine particles was caused by warming and mixing,, and the fatty acids were filtered off through a fat-free filter paper, the flask and paper being washed thoroughly three times with 1 cc. of 5 per cent sodium chloride solution. The filter paper and flask were now extracted with boiling absolute alcohol; the washings were made up to 10 cc. and 1 cc. portions titrated as before.

It was found that this method, which obviously estimates nothing but total fatty acid, gave results consistently lower than the results obtained by the original STEWART and WHITE method. The difference in the results given by the two methods is accounted for if the phosphoric acid of the phospholipins of the blood were present in the alcohol extract, and were titrating as a monobasic acid. In Table IV. are shown the results of fat estimations on samples of blood, by both methods, and also the fat equivalent to the lipoid phosphorus. These figures show how the difference in the results given by the two methods is accounted for by the lipoid phosphoric acid.

The/

The blood-fat estimations, in the experiments described in this paper, were obtained using the original STEWART and WHITE method, and by simultaneously estimating the lipoid phosphorus contents of the bloods, a factor was subtracted in order to obtain the true fat contents. This procedure was found to be more convenient than the use of the modified STEWART and WHITE method, since the lipoid phosphorus contents of the bloods were being estimated in any case, for another reason.

ESTIMATION OF THE LIPOID PHOSPHORUS CONTENT OF BLOOD.

The lipoid phosphorus content of blood was estimated using the method described by BRIGGS (1924). In principle, the method consists of the formation of phospho-molybdic acid and its subsequent reduction by hydroquinone and sulphur dioxide, with the production of a stable blue colour proportional to the amount of phosphorus present. The excess of molybdic acid is not reduced.

REAGENTS.

Molybdate Solution. 5% Ammonium Molybdate in 5N. sulphuric acid. 25 grams of Ammonium Molybdate were dissolved/

dissolved in 300 cc. water, and to this were added 75 cc. concentrated sulphuric acid diluted with 125 cc. water.

Hydroquinone Solution. A 1% solution of hydroquinone to which was added a drop of concentrated sulphuric acid to retard oxidation.

Sulphite Solution. A 20% solution of Sodium Sulphite was used. The strength of the sulphite solution deteriorated on standing, by oxidation, but the amount used in colour development was sufficient, provided an easily detectable smell of sulphur dioxide was evolved.

Stock KH_2PO_4 Solution. This was prepared from pure KH_2PO_4 which had been previously pulverised and dried for several days over concentrated sulphuric acid. 4·394 grams were dissolved in 1000 cc. distilled water, and 5 cc. $CHCl_3$ added as a preservative.

1 cc. = 1 mg. phosphorus.

Standard KH_2PO_4 Solution. 100 cc. Stock Solution were diluted to 1000 cc. with water.

1 cc. = 0·1 mg. phosphorus.

Procedure. 2 cc. of the alcohol ether extract used in the estimation of blood fat, were transferred to a/

a large Pyrex test tube (6 X 1 inches) graduated at 25 cc. and 1 cc. of 10N H_2SO_4, added. A glass bead was dropped in, and the mixture was heated over a micro-burner until fumes of SO_3 began to appear. The tube was allowed to cool for about a minute, and one drop of 30% H_2O_2 (Perhydrol) added. Then the tube was covered with a watch glass to avoid loss of SO_3, and was heated over the micro-burner for about 10 minutes. After cooling, the contents of the tube were diluted with 18 cc. of distilled water. To a similar tube were transferred 0·1 cc. of standard KH_2PO_4 solution, 1 cc. of 10N H_2SO_4, and 17 cc. distilled water. To each tube, were added 2 cc. of the molybdate solution and 2 cc. of the hydroquinone solution. The tubes were covered with small beakers and heated for half-an-hour in a boiling water bath. After cooling, 1 cc. of Sulphite solution was added to each tube, the contents were made up to 25 cc. and, after 15 minutes, the colours were compared in a colourimeter.

Attention must be directed particularly to ensure complete ashing. Heating with sulphuric acid alone was continued until a large amount of charring had taken place. The oxidation of this charred carbonaceous material was very rapid on the addition of the/

TABLE V.

BLOOD SAMPLE	LIPOID PHOSPHORUS	
1.	21.7,	21.9
2.	19.3,	20.0
3.	15.5,	15.1
4.	18.8,	19,0
5.	14.6,	14.2
6.	20.8,	19.2
7.	16.1,	16.3

the hydrogen peroxide, and the subsequent heating for 10 minutes was directed to decompose the excess peroxide, which, if present, would have interfered with the colour production. To ensure complete decomposition of the peroxide, the tubes were heated as strongly as possible without loss of SO_3.

With due care as to ashing this method of lipoid phosphorus estimation gave very good and consistent results, duplicate analyses agreeing to within 5 per cent. being obtained. In Table V. are given examples of the results obtained.

THE ESTIMATION OF TOTAL CHOLESTEROL.

Cholesterol and Cholesteryl Esters were determined in blood by the method of MYERS and WARDELL (1918).

For the estimation, 1 cc. of blood was pipetted on to 4 to 5 grams of Plaster of Paris in a porcelain basin, and dried in a steam oven for 1 hour. The dried mixture was powdered up and placed in a small all-glass extraction apparatus, and continuously extracted with 25 cc. chloroform for 2 hours. The chloroform extract was dried with calcium chloride/

TABLE VI.

BLOOD SAMPLE	CHOLESTEROL
1.	133, 132
2.	156, 156
3.	138, 136
4. (diabetic)	262, 263
5.	118, 114
6.	119, 121

chloride, filtered, and made up to 50 cc., colourimetric estimation being then carried out as follows:- 10 cc. of the chloroform extract were pipetted into a dry test tube, and 2 cc. acetic anhydride and 0·2 cc. of concentrated sulphuric acid were added. 10 cc. of a standard solution of cholesterol were treated in the same way. After thorough mixing the tubes were allowed to stand for 15 minutes in a water bath at 35°C. in the dark, so that the colour was completely developed, then the colours were compared in a colourimeter.

This method estimates total cholesterol in blood and was found to give very accurate results, duplicate analyses agreeing exactly, being obtained. For normal blood, values ranging from 120 mgs.% to 160 mgs.% were found. (See Table VI.)

ESTIMATION OF SOAP IN BLOOD.

In order to estimate the amount of free fatty acids or simple soaps in blood, the method described in 1929 by STEWART and WHITE (1929), was employed. This method attacks, not the fatty acid part of the molecule, which is common to both soaps and/

and fats, but to the metallic part which is peculiar to the soap. The usual alcohol ether extract of the blood was employed for estimating the soap. 2 cc. of blood were added drop by drop to 30 cc. of a mixture of three parts alcohol and one part ether in a 50 cc. flask. The flask was shaken throughout the addition of the blood, and was then heated on the water bath until the ether began to boil. It was then cooled and made up to the mark with alcohol-ether mixture. After thorough rinsing the sediment was allowed to settle and 20 cc. of the supernatent liquor were pipetted off and filtered through a small plug of ether cleansed cotton wool into a silica beaker. The plug was washed with alcohol-ether and the washings run into the beaker. The contents of the beaker were evaporated to dryness on the water-bath (overheating being sedulously avoided) and the residue was thrice extracted with about 1 cc. of hot absolute alcohol. The combined extracts were evaporated to dryness in a hard glass test tube provided with silica clips to prevent bumping, and 0·5 cc. $N/_{350}$ H_2SO_4 was added to the residue. The liquid was cloudy because of the presence of fats and free fatty acids and titration was impossible. To overcome this difficulty 0·5 cc. of/

TABLE VII.

BLOOD SAMPLE	SOAP	
1.	26.8	27.2
2.	36.1	36.8
3.	48.0	48.5
4.	32.7	33.8
5.	29.7	31.6
6.	42.7	44.2

of saturated solution of pure sodium chloride and about 1 cc. of pure light petroleum were added. After standing over night in the ice chest, the liquid cleared and was titrated. Each estimation was carried out in duplicate, and the difference between these titrations and the titration of 0·5 cc. of N/350 sulphuric acid showed the amount of acid neutralised by the metallic part of the soap molecule. The indicator used was brom-thymol-blue.

The results of some specimen analyses and duplicate analyses are given in Table VII.

METHOD for ASCERTAINING the IODINE NUMBER of the TOTAL FATTY ACIDS in BLOOD.

This method is that of McLURE and HUNTSINGER (1928).

Blood was extracted with the usual mixture of alcohol and ether, 5 cc. blood being used for each total volume of 100 cc. 80 cc. of the clear extract were pipetted into a beaker of 250 cc. capacity and evaporated on the steam bath to the volume of a few cc. Then 15 cc. of redistilled alcohol and 0·8 cc. of pure saturated sodium hydroxide solution were/

were added, and the whole boiled for 10 to 15 minutes. Following this period of saponification, evaporation just to dryness was carried out on the steam bath. After cooling, the dry residue was extracted in the cold, with three portions, 20 cc. each portion, of redistilled chloroform. Each portion of the chloroform was poured through a 7 cm. filter paper. This extraction completely freed the residue from cholesterol, and left the soaps behind. The latter were next converted into fatty acids by adding 10 cc. redistilled alcohol, containing 15 per cent of the usual chemically pure 35 per cent hydrochloric acid. The whole was then boiled up for 10 to 15 minutes, after which the acid and alcohol were driven off by evaporation to dryness on the steam bath. The fatty acids were then extracted from the residue in the beaker by three extractions each with 10 cc. portions of cold chloroform. The chloroform portions were decanted through the same filter paper as that used in separating the cholesterol, and were collected in a 100 cc. Enlenmeyer flask. The chloroform solution of fatty acids thus obtained, was evaporated just to dryness. The fatty acids were next redissolved/

redissolved in 1 cc. of chloroform in the flask, and 2 cc. of HANUS' iodine solution were added. The flask was stoppered and its contents mixed by rotation, and then placed in the dark for 1 hour. The unabsorbed iodine was determined by adding 0·5 cc. of a 15 per cent solution of potassium iodide, and 10 cc. of distilled water, and titrating with 0·01 N thiosulphate of sodium solution, starch solution being used as an indicator. Three control flasks, containing 1 cc. chloroform and 2 cc. HANUS' iodine solution were run with each determination. Also, at the same time, the strengths of the HANUS' iodine solution and of the sodium thiosulphate solution were determined by the usual cross titration using a potassium iodate solution. From the weight of the iodine bound by the fatty acid in this procedure, and the weight of the blood fatty acids found by the method of STEWART and WHITE, the iodine number was calculated.

HANUS' iodine solution.

13·2 grams iodine in 1000 cc. of glacial acetic acid with 3 cc. bromine added. The acetic acid must give no green colour with potassium chromate and sulphuric acid.

THE UTILISATION OF FAT BY MUSCLE.

Provided that the hydrogen ion concentration of the blood and tissues is remaining constant, and that no alteration is occurring in the amount of lactic acid present in them, the respiratory quotient is an indicator of the chemical substances undergoing oxidation in the body to provide the energy necessary for the requirements of the body. The use of the respiratory quotient for determining the nature of the fuel used during muscular exercise, demands as a primary condition, that the body shall be in exactly the same condition at the beginning and end of the measured period, except for the loss of fuel. There are many factors which operate to render difficult the utilisation of respiratory data for this purpose. In the intact animal, lactic acid may be removed from the working muscles by the blood stream, and there, it is at once neutralised chiefly by the sodium proteinate buffers present. This tends to raise the hydrogen ion concentration of the blood, and the balance can only be restored by the elimination of carbon dioxide via the lungs. This is aided by the stimulatory/

stimulatory effect of the increased hydrogen ion concentration upon the respiratory centre. When it is remembered that, during the most severe muscular exercise, lactic acid is being set free at the rate of about 3 grams per second, and that this is equivalent to displacing about 0·73 litres of carbon dioxide per second, it can easily be understood how this greatly accelerated output of carbon dioxide gives the respiratory quotient fictitiously high values. The overbreathing always associated with exercise, also increases the elimination of carbon dioxide and raises the value of the respiratory quotient. So that even in moderate exercise, the measurement of the respiratory quotient cannot be expected to give reliable information as to the type of metabolism going on in the body.

After cessation of exercise the respiratory quotient continues to rise at first owing to the carbon dioxide elimination diminishing more slowly than the oxygen intake (HILL, LONG and LUPTON. 1924) When the exercise performed is severe, the production of lactic acid is so rapid that the lungs are unable to wash out the carbon dioxide as quickly as it is liberated, and consequently it accumlates in the tissues/

tissues, with the result that immediately exercise is stopped, the increased elimination of carbon dioxide continues for a short time. Although the oxygen intake falls below its maximum value, it still remains high until all the lactic acid formed during exercise is removed during recovery. Further, as lactic acid is removed, it is necessary for the body to retain carbon dioxide to replace that which was previously eliminated, otherwise the tissues would become excessively alkaline. Consequently, while there is a high respiratory quotient for a short time at the beginning of the recovery period, in the later stages of recovery there is a retention of carbon dioxide as well as a high oxygen intake, and a very low value for the respiratory quotient.

Considering, however, the whole process of exercise and complete recovery, i.e. return to the original values of oxygen consumption, respiratory quotient and blood lactic acid, carbon dioxide content and combining power, it is obvious that the factors which tend to raise unduly the respiratory quotient during exercise, are cancelled by those which tend to lower it during recovery. Even so, however, the conditionsbefore and after exercise may not/

not be strictly comparable, since even moderate exercise involves the excretion of lactic acid, and it is not known how this excretion affects the urinary content of bicarbonate ions or the ammonia-urea ratio. Thus the lactic acid must either be excreted as the sodium or ammonium salt. If it is excreted as ammonium lactate, the lactic acid takes with it, ammonia which would otherwise have been excreted along with carbonic acid, as urea. Therefore, an amount of carbon dioxide equivalent to the lactic acid excreted would be liberated, and its effect upon the respiratory quotient would not be compensated during recovery.

Such factors as these, however, though they may introduce considerable errors in experiments of short duration where the gas volumes dealt with are small, cannot have a very great effect upon the longer experiments about to be described. The particular ones mentioned above, in fact, would tend to make the observed respiratory quotient higher, than the actual ones. Hence, although they, and others of the same kind may afford at any rate a partial explanation of the very high respiratory quotients observed by BEST, FURUSAWA and RIDOUT (1929) in violent exercise/

TABLE VIII.

SUBJECT	WORK DONE kg.m.	BASAL R.Q.	EXCESS OXYGEN LITRES	EXCESS R.Q.	EXCESS and FAT USED in gms. CARBOHYDRATE	FAT	CALORIES PRODUCED per CALORIE OF WORK
NORMAL							
D.M.D.	4300	0.79	17.66	1.04	21.31	---	---
R.G.	5500	0.72	18.14	1.00	21.88	---	---
C.P.S.	15000	0.71	33.83	0.95	33.91	2.85	4.82
J.B.R.	14000	0.77	24.17	0.96	25.25	3.31	5.15
D.S.	18500	0.74	43.03	0.90	34.27	7.27	5.17
R.E.I.	33000	0.75	71.51	0.86	45.30	16.90	4.37
F.P.C.	22000	0.76	49.76	0.92	43.65	6.27	4.75
De V.	38000	0.88	89.66	0.86	56.70	21.56	4.95
R.G.	28000	0.75	62.90	0.86	39.80	15.25	4.67
M.	9000	0.68	18.23	0.96	19.60	1.18	4.23
D.M.D.	5000	0.76	12.50	1.02	15.08	----	5.39

R.G.	6000	0.85	13.92	1.03	16.80	---	5.71
R.M.L.	40700	0.83	88.48	0.88	63.21	17.77	5.40
D.M.D.	25500	0.77	51.28	0.87	34.75	10.94	4.32
M.	13000	0.68	23.69	0.83	12.09	6.96	4.13
J.H.	4000	0.66	9.48	0.97	10.32	0.40	5.09
DIABETICS							
J.McC.	8600	0.72	20.48	0.88	14.64	3.97	4.99
B.	6000	0.80	15.83	0.79	5.43	5.63	5.39
W.McL.	12300	0.81	30.07	0.75	4.51	26.20	5.30

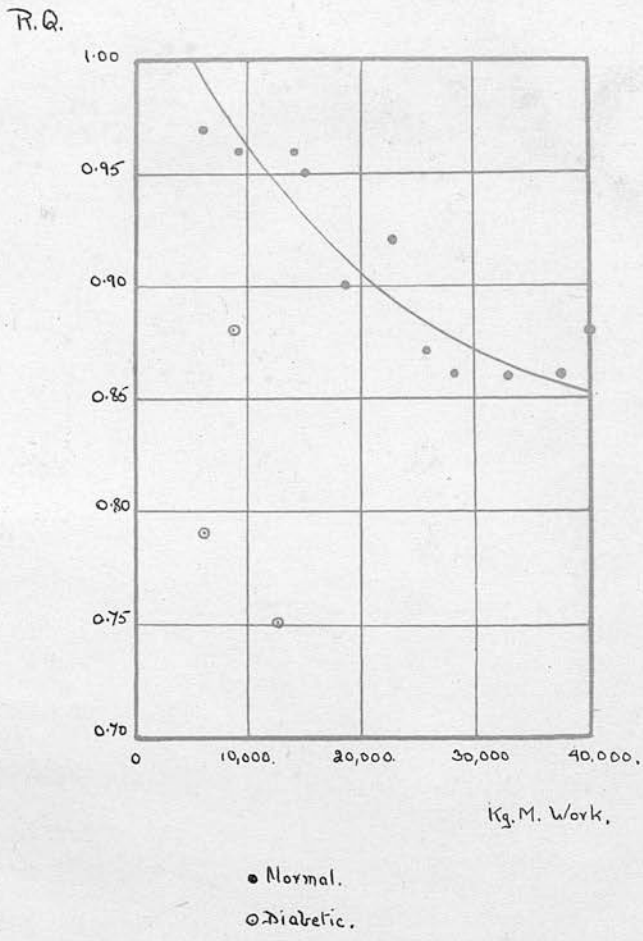
Data from the first two experiments (D.M.D. and R.G.) were not used in the construction of the curves or in calculation of efficiencies. In these experiments work was performed by mangling, and the efficiency, oxygen consumption etc. are therefore not comparable with those of the other experiments. They are given merely to show the respiratory quotient for relatively small amounts of work.

exercise of very short duration, they do not invalidate the deductions drawn here.

All the data obtained from these experiments which are relevant to the points discussed in the following pages, are summarised in Table VIII. The experiments are not arranged in any special sequence, but are tabulated in the order in which they were performed. The excess oxygen consumption over basal requirements, due to the performance of the stated amounts of work, was obtained by subtracting the basal requirement of oxygen during the whole period of exercise and recovery, from the total oxygen consumption. In the same way, the excess carbon dioxide production was obtained and the excess respiratory quotient due to exercise and recovery was calculated. Since the performance of muscular exercise does not increase protein metabolism above the basal level (Table II.) this excess respiratory quotient must be due to the combustion of carbohydrate and fat. From a table published by ZUNTZ and SCHUMBURG (1901), the amounts of carbohydrate and fat consumed during exercise and recovery, may be calculated. Since the respiratory quotient due to the exclusive use of carbohydrate is 1.0, and of fat

0.707/

Figure II.



II. Relationship between the amount of work and the respiratory quotient for exercise and recovery.

0·707, the relative amounts of the two fuels are obtained, and the absolute amounts calculated from the oxygen consumption.

In Figure II the amount of work performed by a number of subjects is plotted against the respiratory quotient for exercise and recovery, at the end of which the oxygen consumption, respiratory quotient, and carbon dioxide combining power of the blood had returned to normal. The figures used in constructing this curve were, of course those for the extra metabolism due to the exercise. The volumes of oxygen utilised and carbon dioxide produced by basal metabolism were subtracted from the total volumes for exercise and recovery, and the resulting figures were these due to the excess metabolism and gave the respiratory quotient for exercise and recovery.

With small amounts of work, (4000-5000 Kg. Ms.) the excess respiratory quotient was at, or very near to 1·0 (in two cases values appreciably higher than this were obtained), but with increasing amounts of work, the respiratory quotient for exercise and recovery steadily fell. In spite of the fact that twelve individuals are represented in the curve, the proportionality/

proportionality between the amount of work performed and the respiratory quotient is definitely marked, and goes far to support the substantial truth of the figures. It is perhaps advisable to reiterate here that the rate at which the different subjects performed the work was nearly constant, the tension on the bicycle ergometer, and the rate of pedalling being kept as nearly constant as possible.

In three cases the respiratory quotient for small amounts of work was very much lower than was expected, a result which indicates, if the conclusions drawn later are justified, a much earlier usage of fat. In these cases there appears to be either a relative inability to utilise glycogen in the normal way or a very low store of glycogen, and it is significant that all three are cases of diabetes mellitus. Since the evidence in the literature (LUSK 1928) indicates that in this condition the muscle glycogen is maintained long after the remaining stores are depleted, and since the cases reported here were under control, it is reasonable to conclude that the fault lay in the utilisation of carbohydrate rather than its lack. It is significant that glycosuria was not produced by the exercise. The conclusion seems/

seems justified that although the muscles prefer carbohydrate as a fuel, they are fairly rapidly forced to fall back on some other fuel of lower respiratory quotient, i.e. protein or fat.

As has already been mentioned, in no case did the results of urine analysis indicate any usage of protein, the excretion of nitrogen remaining unchanged throughout the period of exercise and recovery, and no greater than that of the previous basal period. (Table II.)

Usually, in fact, the urine secreted during the period of exercise and recovery contained rather less nitrogen than that for a corresponding basal period. This slight decrease, no doubt, is to be attributed to an increased elimination of nitrogen by the skin. In this, the experiments confirm the results of other workers.

There remains fat, which, therefore, one is forced to suppose is used by the muscles as a secondary source of energy. The alternative hypothesis, that the extra fat is not used by the working muscles, but is used in the basal processes, thereby liberating carbohydrate for use in the working muscles is disproved by a consideration of the amounts involved in the experiments here described. Thus in one/

Basal respiratory quotient.	0.77
Basal oxygen consumption for 85 minutes.	26.28 litres
Nitrogen excretion during 85 minutes.	1.097 gram.
Carbohydrate used in basal metabolism during 85 minutes.	3.55 gram.
Heat equivalent of Carbohydrate used.	14.86 Cals.
Respiratory quotient for exercise and recovery.	0.96
Oxygen used during exercise and recovery.	24.17 litres
Duration of exercise and recovery.	85 minutes
Calculated fat used in exercise and recovery.	3.31 gram.
Heat equivalent of fat used.	31.35 Cals.

one experiment upon J.B.R., a respiratory quotient of 0·96 was obtained for exercise and recovery, and the relevant data are as follows. This experiment has been chosen for illustrative purposes since, with the small amount of fat usage, it offers a less convincing example than those experiments with lower excess respiratory quotients. (See opposite page.)

These data are obtained from Tables II and VIII. In calculating the carbohydrate used in basal metabolism, it is necessary to obtain from the basal respiratory quotient, oxygen consumption, and nitrogen excretion, the non-protein respiratory quotient. This is found by subtracting from the basal oxygen requirement and carbon dioxide production, those volumes due to the combustion of the observed amount of protein. The factors in the various calculations were obtained from LUSK (1928).

From this calculation it is evident that the energy available from the whole of the carbohydrate used during the period of exercise and recovery would not, if entirely directed to the use of the working muscles, obviate the necessity of their calling upon fat.

The respiratory quotient, though it allows the deduction that fat is used as a fuel by the working muscles, gives no imformation on the far more vexed /

vexed question as to how the fat is used, whether directly, or only by conversion to carbohydrate.

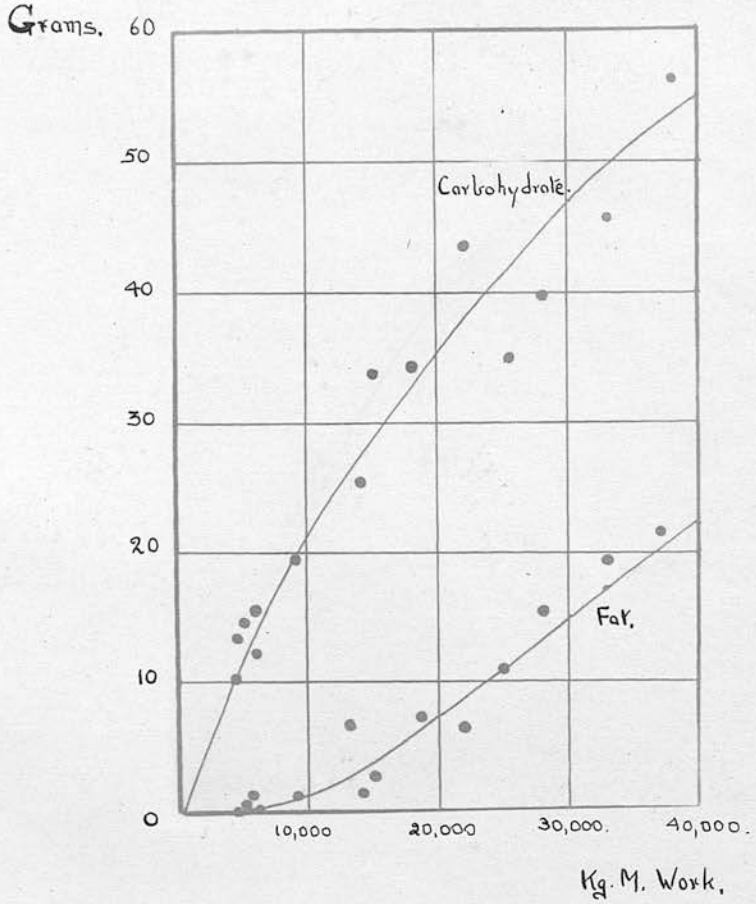
The controversy as to which of these alternative routes is followed by the fat used in muscular exercise hinges largely on the problem of the relative efficiency of fat and carbohydrate. It is rightly argued that if fat is utilised by its direct oxidation, muscular efficiency should probably be unaltered when it replaces carbohydrate; on the other hand, utilisation only of carbohydrate derived from fat necessarily implies a loss of energy and therefore a decreased efficiency. Moreover the loss of efficiency should be the same for every individual. In considering the magnitude of such a loss of efficiency one is faced with the difficulty that the conversion of fat to carbohydrate in the animal body has never been conclusively proved, and that these workers whose experiments have served to demonstrate the possibility of such a conversion, have thrown no light on the mechanism. The smallest wastage of energy, as has often been pointed out, would occur if the whole of the carbon of the fat molecule were converted into carbohydrate, and even then, some 25% of the total energy would be lost.

Such/

Such a complete transformation of fat to carbohydrate is very difficult to picture chemically, though it is intriguing to speculate as to the fate of the pairs of carbon atoms removed in the successive stages of B - oxidation. In the absence of any real knowledge, however, the difficulty of imagining such a change must be ignored, and the most favourable possible mode of conversion must be taken as the criterion in calculating the loss of efficiency to be expected.

The classical work of KROGH and LINDHARD, in which the maximum loss of efficiency was found to be 11%, is often cited as disproving the fat to carbohydrate hypothesis on the ground that greater losses (i.e. 25%) are to be expected. (LEATHES and RAPER, page 197.) The original authors, however, are more cautious, and merely state that their experiments cannot be used as proof that fat is necessarily converted to carbohydrate before being utilised by working muscle. They regarded the usual methods of calculating the loss of efficiency from the conversion of fat to carbohydrate as being too crude, and suggested as a possibility the addition to fat of carbon dioxide and water, a process which would actually/

Figure III.



III. Relationship between the amount of work and the amounts of carbohydrate and fat oxidised.

actually result, they thought in an increase of efficiency - forgetting, as BORSOOK and WINGARDEN (1930) have pointed out, that the carbon dioxide could only be produced at the expense of other reactions.

The change over from carbohydrate to fat as muscular fuel is, of course, not sudden and complete. Even when the fat has become the preponderating source of energy, carbohydrate is still being used, though in diminishing amount. This is shown in Figure III. in which the amounts of fat and carbohydrate used for various amounts of work and recovery are plotted against the amounts of external work performed. The figures for the amounts of fat and carbohydrate are calculated in the usual way from the excess oxygen consumption and respiratory quotient, which has already been described. It is evident from the curve that, as the amount of work increases, the amount of fat used increases, but so also does the amount of carbohydrate. Even with a respiratory quotient of 0·80 carbohydrate is still being used; the respiratory quotient is not simply the mean result of the early exclusive use of carbohydrate/

TABLE IX.

R.Q.	1.0	.95-.99	.90-.94	.85-.89	.80-.84
Efficiency, in Cals. heat produced per Cal. of external work.	4.83 4.76 5.00 5.05 5.39 5.71	4.82 4.23 5.15 5.09	4.32 4.75 5.38 4.80 4.75 5.17	4.11 5.65 4.95 4.67 5.40 4.32 4.37	4.37 4.13 5.04 5.70 4.32 4.06
Mean	5.12	4.82	4.86	4.78	4.60
Distribution. Lowest	4.76	4.23	4.32	4.11	4.06
Highest	5.71	5.15	5.38	5.65	5.70

carbohydrate and the later exclusive use of fat.

The respiratory quotient for a complete period of exercise and recovery can never be that of fat alone except possibly in the case of a complete diabetic; and then only on the assumption that the diabetic cannot oxidise carbohydrate or lactic acid - it must always be either 1·0, or a mean result of the oxidation of both fat and carbohydrate. Hence the loss of efficiency is less, and with values of the respiratory quotient of 0·80 and over, very much less than the calculated 25%. Thus in one of the experiments here described, the respiratory quotient and oxygen consumption showed the utilisation of 56·8 grams of carbohydrate, and 21·56 grams of fat with a respiratory quotient of 0·86. A simple calculation shows the expected loss of efficiency to be only 11·5. In another, with respiratory quotient of 0·92, 43·65 grams of carbohydrate and 6·27 grams of fat were used, and the calculated wastage of energy is 6·2 per cent.

In the experiments being described no loss of efficiency was observed when work was performed at the expense of fat. (Table IX). It is admitted that/

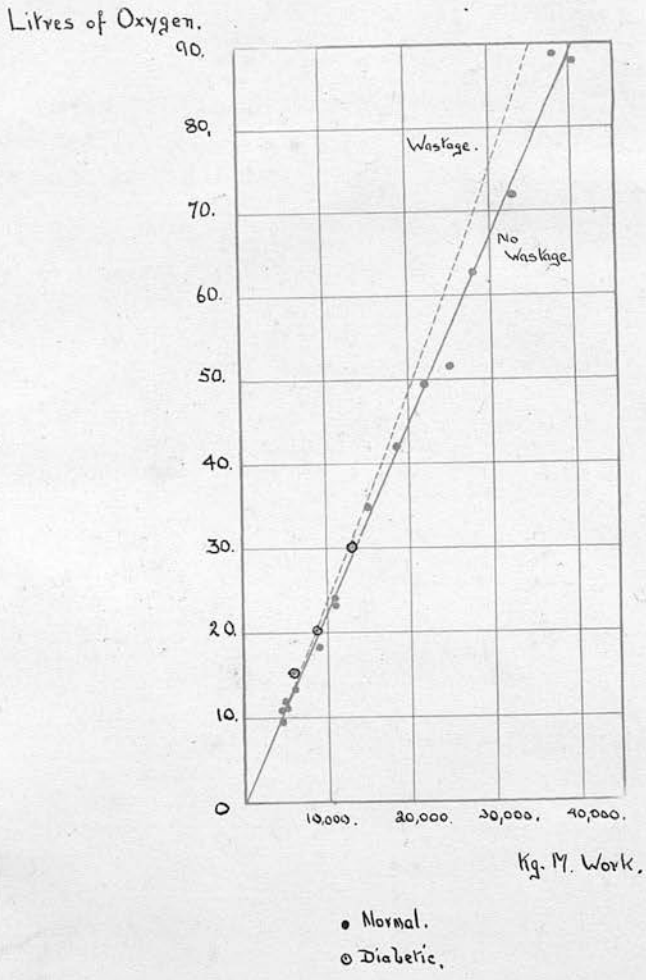
that the experiments were not specially designed for the purpose of showing such a change in efficiency, and that in no case was a series of experiments undertaken upon the same individual with varying amounts of work and at varying respiratory quotients. Their original object was to find if any correlation existed between the amount of fat used in exercise and the change in the blood fat concentration. Nevertheless, since the conversion of fat to carbohydrate is a chemical process, and involves a definite wastage of energy irrespective of individuals, it seems fair to suppose that any such wastage would be shown in the average results from a large number of subjects. Since the subjects were not trained cyclists, the fact that only one experiment was performed upon each, was an actual advantage, for it eliminated any fallacy due to their becoming more accustomed to the form of exercise, and so working with greater efficiency towards the end of the series. The numbers reported here, are not, it is true, large, but as they show no suggestion of wastage of energy with the lower respiratory quotients, and as the distribution in each series, short though it is, is nearly the/

the same, it has been thought worth while to mention them.

They differ radically, of course, from those of KROGH and LINDHARD, and it seems possible that the difference may lie in the fact that all the subjects dealt with here, were on an ordinary mixed diet, while KROGH and LINDHARD used high and low carbohydrate diets to obtain their variations in respiratory quotient. It appears to us that it is a fairer method to vary the respiratory quotient for exercise and recovery by changing the amounts of work performed, rather than by feeding unaccustomed diets which alone might quite well account for difference in efficiency. Moreover, the rate of work in the experiments reported here, was much greater than in those of KROGH and LINDHARD. It might also be conveniently stated here, that it does not appear to be in harmony with other works of nature that the human body, which is constructed to be able to perform enormous quantities of work in emergencies, should do so in a decreasingly efficient manner.

A wastage of energy in the utilisation of fat should become apparent not only in the calculated efficiency/

Figure IV.



IV. Relationship between the amount of work and the oxygen consumption for work and recovery. The continuous line represents the volume of oxygen expected if there is no wastage consequent upon the utilisation of fat. The dotted line represents the oxygen consumption calculated on the basis of a 30% wastage for the conversion of fat to carbohydrate. The points represent the experimentally determined values.

efficiency, but also in another way. The wastage, if fat is converted to carbohydrate, is due to some such process as loss of hydrogen, which is oxidised to water. Thus in the most favourable conversion:-

$$2C_{57}H_{106}O_6 + 47O_2 = 19C_6H_{10}O_5 + 11H_2O.$$
(glyceryl-oleo-stearate)

The utilisation by the muscles of one gram of glycogen derived from fat, demands, according to this equation, the consumption of 1·099 litres of oxygen, instead of the 0·828 litres required by the glycogen itself, - an oxygen wastage of 31·5 per cent. On the other hand, the direct utilisation of fat involves no loss of oxygen, and the curve showing oxygen consumption per calorie of work done, is simply that of the calorific value of oxygen calculated by ZUNTZ and SCHUMBURG (1901). These two curves for the consumption of oxygen per calorie of work done, obtained from these experiments, are shown in Figure IV. The continuous line represents the oxygen used for the direct utilisation of fat, the dotted line that for the conversion of fat to carbohydrate. In those curves allowance is made for the simultaneous use of carbohydrate, the relative amounts of the two fuels being calculated on the basis of Figure III. The experimentally/

experimentally determined values are also shown in Figure IV, and, although there is a certain amount of scattering, due to the employment of many different individuals, it is evident that by far the closer approximation is given by the curve corresponding to direct utilisation of fat. In the curves, as in the table of efficiencies, are included the results from the three diabetic subjects, and it is interesting to note that in spite of their abnormally low respiratory quotients, with, therefore, the utilisation of abnormally high proportions of fat, they show no deviation from the normal efficiency.

Though no finality is, of course, claimed for these results, they tend very definitely to oppose the view of the necessary conversion of fat to carbohydrate prior to its utilisation by the working muscles, and to support the view that fat and glycogen are alternative fuels, although the latter appears to be used preferentially.

The theoretical need for a conversion of fat to carbohydrate has, indeed, been greatly weakened by the recent work on creatine phosphoric acid, and the discovery of the ability of muscle to work when all production of lactic acid has been inhibited by/

by poisoning with iodoacetic acid, which appears to show how the break down of glycogen to lactic acid can no longer be regarded as essential in muscle contraction. Even if the glycogen-lactic acid cycle be considered essential, it may well be that fat, by its direct oxidation, is capable of sparing lactic acid, leaving more for resynthesis to glycogen, and so conserving the supplies of carbohydrate. Pointing in this direction is the finding, in severe diabetes, that muscular exercise involves no oxidation of carbohydrate. (GRAFE and SALOMON. 1922), that the muscles nevertheless hold tenaciously to glycogen (RINGER, DUBIN, and FRANKEL, 1921), and that in partial diabetes at any rate, exercise involves the breakdown of glycogen to lactic acid just as in normal cases. (HIMWICH, LOEBEL and BARR, 1924.)

It is perhaps worth pointing out that if the diabetic organism cannot oxidise carbohydrate, and cannot convert fat into carbohydrate, (the evidence concerning which has already been discussed,) and that fat metabolism is the same in the diabetic as in the normal organism as far as hydroxybutyric acid, then if the normal individual does convert fat to carbohydrate he/

TABLE X.

SUBJECT	EXCESS R.Q.	EXCESS OXYGEN LITRES	GRAMS GLYCOGEN USED	GRAMS FAT USED	CALORIES of WORK OBSERVED	CALORIES of WORK CALCULATED 1.	CALORIES of WORK CALCULATED 2.
C.P.S.	0.95	33.83	33.91	2.85	35.1	34.6	29.9
J.B.R.	0.95	33.75	33.75	2.86	32.7	34.6	29.9
D.S.	0.90	43.03	34.27	7.27	43.3	43.6	31.3
P.E.J.	0.86	71.51	45.30	16.90	77.2	71.8	43.2
F,P.C.	0.92	49.76	43.60	6.27	51.5	50.6	39.1
De V.	0.86	89.66	56.70	21.56	88.9	89.0	54.2
R.G.	0.86	62.90	39.80	15.25	65.5	63.7	38.0
M.	0.96	18.23	19.60	1.18	21.1	18.8	17.2
D.M.D.	1.02	12.50	15.08	-	11.7	13.0	-
R.G.	1.03	13.92	16.80	-	14.0	14.4	-
R.M.L.	0.88	88.48	63.21	17.77	95.2	89.1	58.8
D.M.D.	0.87	51.28	34.75	10.94	59.7	51.5	32.6
M.	0.83	23.69	12.09	6.96	30.4	23.6	12.1
J.H.	0.97	9.48	10.32	0.40	9.4	10.2	9.0

The Mean efficiency is $\frac{1}{4.87}$

he must do so after the stage of hydroxybutyric acid has been reached. In other words, of a fat molecule containing some fifty-three atoms of carbon, only twelve, or fifteen if the glycerol is included, can be available for glucose synthesis. But if hydroxybutyric acid goes to sugar, the most probable course is:- first oxidation to acetaldehyde which can then undergo aldol condensation. Hence of fifty-three atoms, nine may appear as carbohydrate.

In these experiments now being described, sufficient data is furnished to enable calculation to be made of the amount of external work which could possibly be accomplished (i) when fat is oxidised directly and spares lactic acid, and (ii) when fat is first converted to glycogen, and the glycogen alone is used.

The results of these calculations in a considerable number of cases, show that the observed and calculated amounts of work agree closely when fat is taken to be directly oxidised, but that the observed amount of work could not possibly have been done at the expense of glycogen derived from fat. These results are summarised in Table X.

In/

In calculating the amounts of work possible by each method of utilisation of fat, the mean efficiency of all the experiments was used for each subject. Since a considerable variation in efficiency was found, this accounts for the fact that in one or two of the experiments shown in Table X. a greater amount of work appears to have been done, than would be possible on the observed amount of fuel burned. If the particular efficiency for each subject had been used instead of the mean value, this, of course, would not have occurred. It is important that, without exception, the amount of work performed could not possibly have been done at the expense of glycogen derived from fat. It will be noted, that when the amount of work performed is small, the difference between the two calculated amounts of work is not marked, but with greater amounts of work, owing to the increased utilisation of fat, the difference becomes very considerable.

TABLE XI.

SUBJECT	WORK kg.ms.	BLOOD SAMPLE	FAT mgs.%	CHOLESTEROL mgs. %	LIPOID P. mgs.%	SOAP mgs.%
A R.G.	5500	a.	492	134	-	40.0
		b.	433	139	-	37.9
		c.	481	128	-	41.2
		d.	-	133	-	41.2
C.P.S.	14966	a.	689	136	21.7	27.9
		b.	693	136	21.9	17.6
		c.	522	137	21.6	24.6
		d.	562	137	20.0	27.2
D.S.	18564	a.	478	143	20.7	-
		b.	453	144	22.3	-
		c.	450	125	23.1	-
		d.	481	127	20.3	-
R.E.I.	19123	a.	607	118	13.3	30.7
		b.	791	114	12.1	30.2
		d.	583	118	11.6	31.2
R.G.	28050	a.	480	145	-	30.7
		b.	588	144	-	45.7
		d.	434	146	-	39.8
R.E.I.	33000	a.	511	118	18.8	23.9
		b.	674	118	16.5	25.2
		c.	467	-	19.0	25.0
		d.	425	-	15.0	23.9
De V.	37150	a.	376	120	15.3	23.6
		b.	511	142	15.2	24.6
		c.	464	127	17.2	24.1
		d.	460	122	17.5	24.5

B	D.M.D.	4300	a.	853	208	21.7	28.2
			b.	685	209	21.9	26.7
			c.	522	208	21.8	22.6
			d.	562	207	21.7	27.2
	J.B.R.	14000	a.	1103	139	15.5	29.7
			b.	831	141	18.3	35.7
			c.	694	140	15.1	42.7
			d.	899	139	14.5	42.7
C	J. McC.	8600	a.	770	-	-	-
			b.	825	-	-	-
	W. McL.	12300	a.	690	-	-	-
			b.	812	-	-	-
D	F.P.C.	22250	a.	310	119	15,9	36.0
			b.	660	121	15.5	34,7
			c.	518	121	-	35.7
			d.	410	120	15.6	35.2

a = Before Work . b = After Work

c = After Partial Recovery. d = After Complete Recovery.

THE VARIATIONS IN BLOOD FAT IN MUSCULAR EXERCISE.

The results of blood analysis (Table XI) show that the majority of normal subjects do experience some increase in the blood fat, when, under fasting conditions, they perform work in excess of approximately 8000 to 10,000 kilogram metres. This figure is very approximate, since the exact point at which the rise in blood fat occurs depends to a considerable extent on the rate at which the work is performed. Table XI gives typical results of blood analysis. The experiments on normal subjects (A) show that the rise in blood fat does not occur in every case (e.g. C.P.S., D.S.), when it does occur, it is transient, and has disappeared at the end of the recovery period.

PATTERSON (1926) observed that diabetic subjects almost invariably showed a fall in the blood fat, even with the relatively small amounts of work they were able to perform. All his diabetic subjects, however, had initially, an abnormally high blood fat, a state of affairs which is usually present in this disease. The experiments summarised in Table/

Table XI B show, however, that this fall in the blood fat produced by exercise, is characteristic, not of diabetes mellitus, but of the incidental high blood fat level. It occurred in a number of subjects whose fasting blood fat was abnormally high, but who were, apparently, healthy in all other respects. Moreover, in two diabetic cases (Table XI C), with basal blood fat not much above the normal level, exercise produced a small, but definite increase, more easily than in normal subjects. Nor, probably, is it mere coincidence, that in one case the converse appeared to hold, and that a healthy subject with an abnormally low fasting blood fat level showed an abnormally great increase after a moderate amount of work (Table XI D). These cases lead to the conclusion that the blood fat content tends to reach the normal level before it shows the increase usually given by healthy individuals. This preliminary change takes place under the influence of moderate work, definitely below the amount required to produce a change in the blood fat content of subjects whose basal blood fat is within the normal range.

These experiments in which the subject performed a second period of work after recovery from the/

TABLE XII.

SUBJECT	BLOOD SAMPLE	WORK kg.m.	BLOOD FAT mgs.%	BLOOD CHOLESTEROL mgs.%	BLOOD LIPOID PHOSPHORUS mgs.%
R.G.	Basal	----	611	145	14.6
	After 1st work	28000	719	144	14.2
	After recovery	----	565	146	14.6
	After 2nd work	11000	619	147	14.3
R.E.I.	Basal	----	607	118	13.3
	After 1st work	19000	791	114	13.1
	After recovery	----	583	118	11.6
	After 2nd work	9000	647	118	13.7

the first, demonstrate (Table XII) that a rise in the blood fat is more easily obtained during the second period than during the first. This point is of importance as emphasising the necessity of obtaining basal condition before starting the exercise if comparable results are to be obtained from a series of experiments on normal individuals. It also tends to support the contention that the increase in blood fat during exercise is in some way connected with the utilisation of fat by the muscles. There can be no doubt that at the commencement of the second period of exercise the body stores of glycogen have been somewhat depleted, and hence the muscles - if they can use fat - must call for fat earlier, though only slightly so, than in the first period. It is at least a striking coincidence.

The results so far described confirm the statement that muscular exercise is accompanied by an increase in the blood fat, though they show that this is not an invariable result. It was desired however, to find in what constituents of the total blood fat this increase occurred. The analyses (Tables XI & XIII) showed the increase lay solely in the triglycenide fraction which, under resting conditions/

TABLE XIII.

SUBJECT	TOTAL CHOLESTEROL	FAT REQUIRED TO ESTERIFY COMPLETELY	RISE IN FAT
R.E.I.	118 mgs. %	13.7 mgs. %	163 mgs. %
FF.P.C.	121	14.0	350
De.V.	142	16.5	135
R.G.	144	16.7	108
R.E.I.	114	13.2	184
R.M.L.	136	15.8	92
R.G.	129	15.0	220

conditions, forms a very small part of the total blood lipoids (CHANNON and COLLINSON 1929). While the total fatty acids increased, the lipoid phosphorus and the cholesterol showed, either no change or only small inconstant variations. The free fatty acid (soap) content of the blood remained unaltered, and though there was possibly an increase in the amount of esterified cholesterol, any such increase was quite incapable of accounting for the observed increase in the total fatty acids. Figures in support of this statement are given in Table XIII., which is based on the ratio of esterified to unesterified cholesterol given by CHANNON and COLLINSON (1929). Finally since PATTERSON'S figures were obtained by the use of the original STEWART and WHITE method it is important to note that exactly similar results are obtained as in the experiments here described, by use of the modified method, which estimates only fatty acids and does not include phosphoric acid. Otherwise the apparent increase in the blood fat during exercise might conceivably have been regarded as due merely to an altered state of dissociation of the phospho-lipins.

A determination of the iodine number of

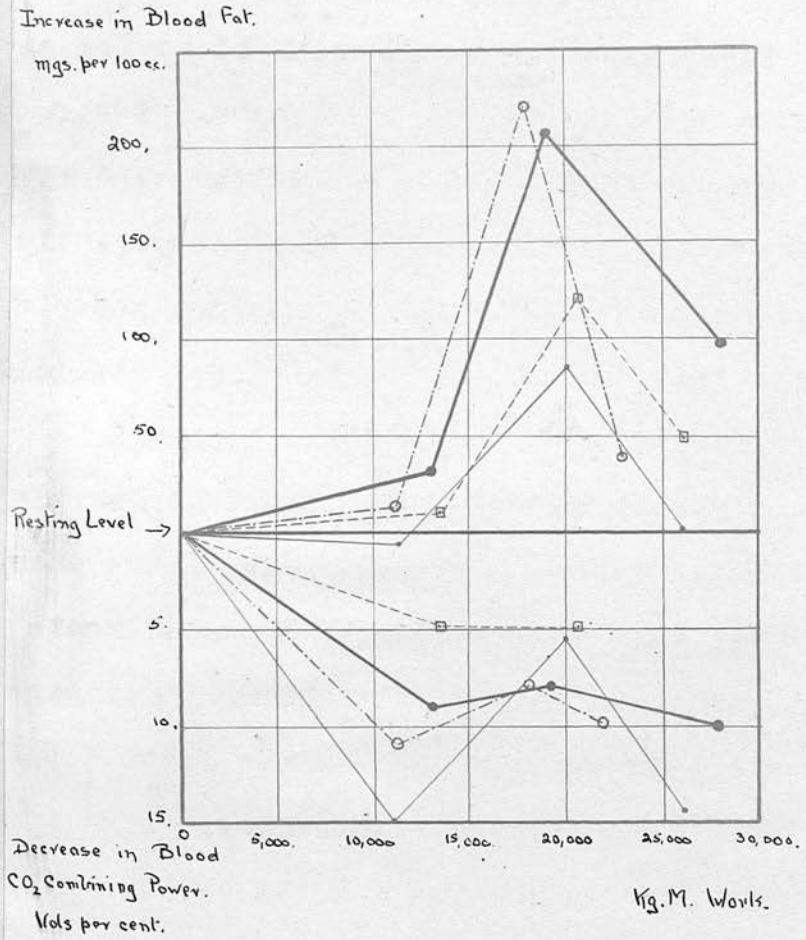
TABLE XIV.

SUBJECT	WORK	FAT	IODINE NUMBER	SUBJECT	WORK	FAT	IODINE NUMBER
T.F.	BASAL	426 mgs. %	63	E.B.H.	BASAL	364 mgs.%	26
	11550 kg.ms.	421	65		13250 kg.ms.	374	24
	16885	661	52		20500	485	14
	24650	448	32		26650	387	26
E.G.	BASAL	660	84	A.T.B.	BASAL	444 mgs.%	22
	13750 kg.ms.	690	65		10250 kg,ms.	454	27
	18965	865	62		16250	454	34
	28000	757	78		23050	444	36

the blood fat before and after exercise, might be expected to give information regarding the source of the extra fat poured out into the blood stream during prolonged muscular exercise. If the fat is being withdrawn from the liver, an increase in the degree of unsaturation is to be expected since the liver fat is highly unsaturated. On the other hand, the fat in the adipose tissue consists principally of a mixture of oleic and stearic glycerides, so that it is relatively less unsaturated than liver fat, and contains about the same amount of unsaturated fatty acid as does the blood fat itself. Mixture of fat from adipose tissue with the blood fat would therefore have little or no effect upon the iodine number.

The iodine number of the blood fat before and after exercise was found in a number of cases (Table XIV), and although it was found to be very variable for different subjects, it altered only very little in the same subject with exercise, and in no case did it show a rise. This suggests very strongly that the source of the blood fat poured into the blood stream in response to stimulation by exercise, is the adipose tissue and not the liver.

Figure V.



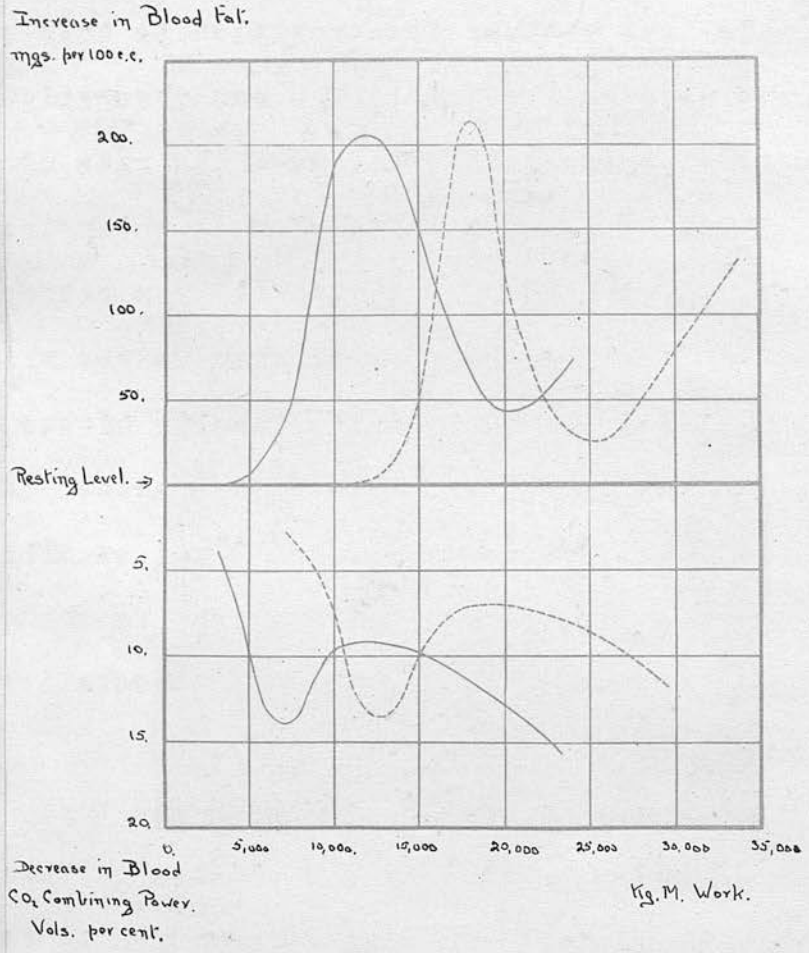
V. The blood fat and the carbon dioxide combining power during exercise. Individual cases.

THE VARIATION OF THE BLOOD-FAT DURING MUSCULAR EXERCISE.

Those experiments in which blood fat estimations were made during the performance of work showed a curious result which ultimately shed light on the marked individual variation and apparently hap-hazard responses obtained in previous experiments. It appeared that instead of the blood fat steadily increasing during exercise, it first rose and then fell. This result was obtained in a considerable number of individuals, and typical cases are shown in Figure V. When the results of 16 experiments were plotted together as a composite curve, it appeared that actually there was a rise in the blood fat followed by a fall and then a second rise. (Figure VI, dotted curve). Moreover the results obtained from nearly all the previous experiments could be fitted to this curve. Certain exceptions were noted, as when the rate of work differed markedly from that usually employed, or when the subject had an abnormal resting blood fat.

The shape of the composite curve had been disguised in the later individual experiments shown in/

Figure VI.



VI. Composite curves showing changes in the blood fat concentration and the blood carbon dioxide combining power during exercise. The dotted line represents changes at 900 Kg.M. per minute; the continuous line changes at 11 00 Kg.M. per minute.

in Figure V by the fact that only three points were obtained. To test whether the exception to this composite curve were really due to the one observed variation in experimental technique - the rate of work - a second series was undertaken in which the subjects worked at a rate of 1,100 kilogram metres per minute, instead of the 900 kilogram metres which had previously been the rule. The results of the fat determination from this series fell on a second composite curve (smooth line in Figure VI) of exactly the same shape as the first, but showing the changes in the blood fat concentration to take place after a smaller amount of work than previously.

These variations in the blood fat were, of course, entirely unexpected, and could not be correlated with a steadily increasing consumption of fat by the working muscles. Yet experiment had shown that such a steadily increasing consumption of fat did indeed exist. (Figure III). It became necessary, therefore, to discard the simple suggestion that the increase in blood fat during exercise was a direct response to a demand by the muscles for an alternative fuel, consequent upon the utilisation of glycogen. Attention was thus directed to the discovery of some stimulus which could act as a connecting link between the/

the two phenomena. The production of lactic acid had suggested itself as a possible causative factor in the rise of blood fat, which at that time, was all that had been observed. For ease of measurement, the carbondioxide combining power of the blood had been determined in all samples. The results of these analyses showed at first somewhat irregular decreases in the carbon dioxide combining power of the blood during exercise, but it was only when blood samples were analysed during the course of exercise that any real relationship between the blood fat and the carbon dioxide combining power was observed. It then transpired that the carbon dioxide combining power, instead of falling steadily with increasing amounts of work to the acidosis level, followed a course which was roughly the reciprocal of that followed by the blood fat. (Figure V) A composite curve of all the results obtained, just as in the case of the blood fats, was sinuous, showing a fall, followed by a partial recovery, and only then a steady progression to the acidosis level. (Figure VI).

The second series of experiments in which blood samples were drawn during the progress of exercise, showed a composite curve of exactly the same character as the first (Figure VI), again, however, as in the case of the blood fat concentration the/

the fall occurred at an earlier period of the work. Moreover, in both series the fall in the carbon dioxide combining power preceded the rise in blood fat, and its partial recovery preceded the fall in blood fat. It is impossible at present to say whether this time relation between the curve of carbon dioxide combining power and that of the blood fat concentration has any real significance. It is none the less suggestive that it indicates a causative relationship between the two phenomena. Formation of lactic acid with consequent depletion of the alkali reserve of the blood would indicate a depletion of the glycogen stores of the body. It might well be that the slight change in the hydrogen ion concentration of the blood thus produced, would stimulate the pouring out of fat from the depots. Since the fat in the depots exists as a water in oil emulsion, and that of the blood as an oil in water emulsion, any slight variation in hydrogen ion concentration would be sufficient to alter the equilibrium between the two systems. Knowledge on this subject is at present so slight that to pursue this hypothesis further without experimental evidence, would be unprofitable. It is, none the less, an interesting/

interesting speculation.

No explanation is, as yet, forthcoming to account for the temporary recovery in the carbon dioxide combining power of the blood as exercise progresses. Prior to this, this phenomenon has not been described. It seems improbable that its cause is due to a decreased production of lactic acid, though, of course, this is not impossible. More probably it may be due to the shift of chloride ions from the plasma into the corpuscles. Such a shift is known to take place under other circumstances (VAN SLYKE 1921) and is of an order of magnitude sufficient to account for the change in alkali reserve found in these experiments.

Further, if the rate of work be greater than about 1,200 kilogram metres per minute, the preliminary temporary changes in the blood carbon dioxide combining power do not occur and the well known acidosis level of exercise is quickly reached.

SUMMARY.

I. When normal healthy men performed muscular work on an ergometer bicycle at rates varying from about 800 to 1,200 kilogram metres per minute, the blood fat usually rose after about 8,000 kilogram metres of work had been done.

II. The appearance of the increase in the blood fat concentration appeared earlier with greater rates of work.

III. After recovery from a first period of work, a second period produced a rise in the blood fat more easily than usual.

IV. A high fasting blood fat in normal or diabetic subjects led to a preliminary fall when work was performed; an abnormally low blood fat was increased by relatively small amounts of work.

V. This alteration in the blood fat was confined to the simple glyceride fraction, and the extra fat was probably derived from the adipose tissue.

VI. Continuance of work led to a return of the blood fat towards normal (at about 18,000 to 21,000 Kg. M.), and later to a second rise.

VII. The carbon dioxide combining power of the blood during muscular work of this type followed a course which was roughly the reciprocal of the blood fat, but the changes in the carbon dioxide combining power preceded those in the blood fat.

VIII. The respiratory quotient for exercise and recovery was unity for amounts of work up to about 5,000 Kg. M., and thereafter fell steadily with increasing amounts of work.

IX. Analysis of the respiratory quotient, oxygen consumption, and nitrogen excretion, showed that protein was not used for work, that carbohydrate continued to be used throughout, though in decreasing amounts, and that fat was used in increasing amounts.

X. There was no relationship between the utilisation of fat and the changes in the blood fat concentration.

XI. No diminution in efficiency was observed at the lower respiratory quotients, and no wastage of oxygen occurred.

XII. The figures obtained enabled several calculations to be made concerning the mode of utilisation of fat in muscular exercise, but in no way did they suggest that fat is converted into carbohydrate.

XIII. Diabetic subjects, though performing work at a lower respiratory quotient than normals, showed no less efficiency, and no wastage of oxygen.

XIV. A modification of the STEWART and WHITE (1925) method for the estimation of blood fat is described.

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The Metabolism of the Frog's Isolated Heart.

By Professor A. J. Clark, M.D.,
C. P. Stewart, M.Sc., Ph.D., and R. Gaddie, B.Sc.

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The metabolism of the frog's isolated heart. By A. J. Clark, R. Gaddie and C. P. Stewart.

The respiratory quotient of the frog's isolated heart was measured. A modified Barcroft manometer was used similar to that described by Clark and White (J. Physiol. 66. 185. 1928); 4 c.c. *M*/20 NaOH was placed at the bottom of the bulb, the changes in conductivity were measured, and the carbon dioxide production was thus calculated. In addition the following chemical estimations were made: glycogen content of hearts (modified Slater and Kermack method); sugar content of perfusion fluid (Hagedorn-Jensen method); nitrogen content of perfusion fluid (micro-Kjeldahl method); fat content of heart (Stewart and White method).

In a series of experiments in which the hearts were perfused with Ringer's solution for 24 hours the following average figures were obtained: total oxygen use about 3 c.c.; respiratory quotient 0·84. The glycogen content after perfusion was 0·2 p.c., which is about one-third of the normal content. This corresponded to a glycogen loss of about 0·5 mgm., and about 0·4 mgm. sugar passed into the perfusion fluid. In some cases the sugar excreted was greater than the estimated glycogen loss. The actual glycogen loss corresponded to an oxygen consumption of less than 0·4 c.c., which is about one-eighth of the oxygen consumed by the heart. About 0·3 mgm. nitrogen was excreted which corresponded to an oxygen consumption of nearly 2 c.c. The fat content of perfused hearts (4·73 p.c.) was nearly the same as the fat content of control hearts (4·84 p.c.). Hearts perfused with frog's serum showed respiratory quotients and glycogen losses similar to those shown by hearts perfused with Ringer's solution.

The chemical analyses and the measurements of the respiratory quotient together indicate that less than one-quarter of the material oxidized by the frog's isolated heart is carbohydrate. No evidence has been obtained for the oxidation of fats by the heart, but there is evidence that protein is broken down, and in some cases the figures suggest that the heart can form sugar from protein.

XXII.—The Metabolism of the Frog's Isolated Heart.

By Professor A. J. Clark, M.D., C. P. Stewart, M.Sc., Ph.D., and R. Gaddie, B.Sc.

(MS. received July 22, 1930. Read July 7, 1930.)

THEORIES regarding the chemistry of the contraction process in striped muscle have been revolutionised during the last year. The Hill-Meyerhof theory was based on the assumption that the contraction process was due to the liberation of lactic acid by the break-down of glycogen, a non-oxidative reaction, and that the recovery process consisted in the oxidation of part of the lactic acid and the resynthesis of glycogen from the remainder. Meyerhof now believes (1, p. 305) that contraction is produced by the break-down of creatin-phosphoric acid (phosphagen), and that the energy produced by carbohydrate break-down is utilised to resynthesise phosphagen.

The two most important implications of this theory are as follows: In the first place, the break-down of phosphagen to creatine and phosphoric acid must result in increased alkalinity. In the second place, since carbohydrates are assumed to play a secondary rôle in the contraction process, there is no reason why other substances should not act equally well as sources of energy, hence this theory of the chemistry of muscular contraction provides no *a priori* reason for assuming that fats and proteins are converted to carbohydrates before being utilised as sources of energy for muscular contraction. This new theory of muscular contraction has been derived from experiments on skeletal muscle, and it is interesting to consider its application to the contraction process in cardiac muscle.

THE CONTRACTION PROCESS IN THE HEART.

The following facts have been established regarding the energy exchange of heart muscle:—

(1) The amount of oxygen consumed and energy released are determined chiefly by the initial length of the heart muscle fibres. An approximately linear relation between heart volume and oxygen consumption has been demonstrated in the frog's heart (Clark and White (2, 3), Eismayer and Quincke (4)), and in the dog's heart-lung preparation (Starling and Visscher (5), Hemingway and Fee (6)).

(2) The resistance against which the heart contracts has relatively little influence on the oxygen consumption; but the heart is not a convenient preparation on which to measure accurately such relations (Clark and White (3)).

(3) The potential energy released by the contraction process is somewhat less than half the energy equivalent of the oxygen consumed (Clark and White (3)).

(4) The contraction process in the heart can occur in the absence of oxygen, for many workers have shown that a frog's heart, if deprived of oxygen but perfused with alkaline solution, will continue to function for many hours (Bachmann (7)).

(5) The heart muscle when it contracts under anaerobic conditions forms lactic acid, and this acid, if it is allowed to accumulate, paralyses the heart (Katz and Long (8), Redfield and Medearis (9), Arning (10), Boyland (11)).

These results are in accordance with the facts known about the energy exchange in skeletal muscle, and suggest that the fundamental contractile process in the two types of muscle is similar.

Experiments by Clark and White (2 and 3) showed, however, that cardiac muscle differs from skeletal muscle in at least two important respects.

(1) The resting metabolism of cardiac muscle is about one-fifth of the metabolism during moderate activity. It amounts to about 0·5 c.c. oxygen per gram per hour, which is about twenty times the corresponding figure for frog's skeletal muscle (Meyerhof (12)). This difference is one which is to be anticipated from the difference in function of the two types of muscle. Most skeletal muscles often remain at rest for many hours at a time, and hence a low resting metabolism in skeletal muscles represents an important economy for the body. The heart, however, never rests, therefore restorative processes must proceed continuously, and hence a high resting metabolism is to be expected.

(2) Cardiac muscle is inhibited by acid much more easily than is skeletal muscle. This difference explains why the heart has less power than skeletal muscle to incur oxygen debt. There is adequate evidence that acid metabolic products are formed in absence of oxygen, and that these rapidly paralyse the heart, whereas skeletal muscle can contract in presence of much higher concentrations of acid.

With regard to the chemistry of the heart, this contains a considerable store of glycogen (0·3 to 0·5 per cent.) and a significant content of other carbohydrates (Boyland (11)). The heart contains a relatively large amount of organic phosphates, and its lecithin content is higher than that of

most other tissues, but the phosphagen content of heart muscle is only about one-tenth that of skeletal muscle (Eggleton and Eggleton (13)). This low phosphagen content makes it probable that the activity of heart muscle depends primarily on some other compound. Meyerhof (14) already has shown that the contraction process in crustacean muscles depends not on phosphagen, but on arginin phosphoric acid.

The Metabolism of the Frog's Heart.

The authors studied the metabolism of the isolated heart of the frog under varying conditions. The oxygen uptake and carbon dioxide consumption were measured, and analyses of the hearts and of the perfusion fluids were made (Clark, Gaddie, and Stewart (15)). We commenced with the simplest system, namely, the heart perfused with a few c.c. of Ringer's solution which contained no glucose. We found that the heart lost carbohydrates, but that part of these were excreted into the Ringer's fluid, and that the amount oxidised was far too small to account for the total oxygen consumption of the heart.

Control experiments showed that the average content of fresh frogs' hearts was: glycogen 0·55 per cent., total carbohydrates 1·3 per cent. After 24 hours' perfusion the glycogen was reduced to about one-third, and the total carbohydrates to about one-half. These figures indicate that a frog's heart of 0·2 gm. lost about 1·3 mgm. carbohydrate during 24 hours' perfusion. About 0·4 mgm. carbohydrate could, however, be recovered from the perfusion fluid, and therefore the net carbohydrate loss was about 0·9 mgm. This quantity of carbohydrate corresponds to an oxygen consumption of about 0·6 c.c. The heart, however, used from 4 to 5 c.c. of oxygen during 24 hours' perfusion.

The heart must therefore burn either fat or protein. The analysis of fat in small amounts of tissue presents great technical difficulties, but our analyses have failed to show any difference in the fat contents of hearts or of serum perfused through the hearts before and after perfusion.

Analysis of the perfusion fluids showed that nitrogen was excreted and that the nitrogen excretion persisted after many hours' perfusion. This last point is important, because an initial nitrogen excretion might have been due to the washing out from the heart of soluble nitrogen compounds. The total nitrogen excreted during 24 hours was about 0·33 mgm. The protein equivalent of this quantity of nitrogen would be oxidised by about 2 c.c. of oxygen, which is about one-half of the quantity of oxygen actually used by the hearts. Continuous measure-

ments of the respiratory quotient over 24 hours showed that the respiratory quotient was about 0·82. This value agrees well with the hypothesis that the isolated heart burns chiefly proteins together with a small amount of carbohydate.

It is of course possible that the proteins are converted to carbohydrate and are used in this form, but our experiments do not provide evidence either for or against this hypothesis.

Carbohydrate Metabolism in the Heart.

Starling and Evans (16) found that the isolated heart-lung preparation of the dog used relatively little carbohydrate, for its R.Q. was as low as 0·85. These experiments were done before the discovery of insulin, and much recent work has been done to determine the influence of insulin on the sugar consumption and on the R.Q. of the heart-lung. The average oxygen consumption of the heart-lung preparation was about 3 c.c. per gram per hour, which is equivalent to 4·3 mgm. of glucose per gram per hour.

Cruickshank (17) made extensive experiments on the sugar consumption of the normal and diabetic hearts of the dog. When no insulin was present, *i.e.* when a diabetic heart was perfused with diabetic blood, he found that there was practically no sugar usage. When a normal heart was perfused with normal blood the sugar usage was only 2 mgm. an hour, but when excess of either insulin or sugar was added, the sugar usage rose to about 5 mgm. an hour, a quantity which corresponded to the total oxygen consumption; and finally, when excess of both insulin and glucose were present, the sugar used was from 8 to 12 mgm. per hour. In this latter case there also was evidence of glycogen storage.

Bayliss, Muller, and Starling (18) studied the influence of insulin on the R.Q. and showed that addition of excess of insulin and sugar caused the R.Q. to rise to between 0·9 and 0·95. These results indicate, therefore, that the consumption of glucose by the tissues more or less follows the law of mass action and is increased when the insulin and the glucose concentration in the perfusion fluid are both increased. When the amount of sugar taken up is greater than the amount which the heart can consume, then the excess is stored as glycogen.

These experiments show that under certain conditions the mammal's heart can use glucose freely, but it is necessary to emphasise the fact that the conditions needed are abnormal. The quantities of insulin introduced in the experiments mentioned were high enough to have produced hypoglæmic convulsions in the intact animal, and the quantities of sugar

perfused were far above the normal and in the intact animal would have produced glycosuria. These experiments, therefore, do not provide much direct evidence regarding the behaviour of the normal heart.

We have not yet found the conditions necessary to enable the isolated heart of the frog to oxidise carbohydrates in preference to proteins. Hearts have been perfused with the following mixtures: Ringer's fluid plus glucose (50 to 100 mgm. per cent.); Ringer's fluid plus glucose and insulin; Ringer's fluid and frog's serum plus glucose and insulin. (The blood-sugar content of frog's serum was found to be about 40 mgm. per cent.)

The glycogen content of the heart was found to decrease rapidly during the first six hours' perfusion, and we have as yet found no means of checking this break-down. Analysis of the perfusion fluids also showed little evidence for sugar consumption. Under no conditions did the sugar usage appear to rise above about 0·03 mgm. per heart per hour. This corresponds to an oxygen use of about 0·02 c.c. per heart per hour, whereas the actual oxygen consumed was about 0·2 c.c. A study of the R.Q. before and after the addition of glucose and insulin to the perfused heart also gave uncertain results. The R.Q. appeared to rise slightly (from 0·85 to 0·9), but no marked change occurred.

The isolated frog's heart is therefore less readily affected by the presence of excess of glucose and insulin than is the isolated dog's heart. Possibly the difference is due to a difference in the time relation. Excess of insulin in the dog can produce hypoglycæmic convulsions in a couple of hours, whereas this effect is only produced after 24 hours in the frog. The fact, however, that the frog's heart continues to beat in such a satisfactory manner without using much carbohydrate does suggest that the heart under normal conditions probably oxidises not only carbohydrates but also other substances.

In addition, we noted that the addition of glucose to the perfusion fluid did not increase the total oxygen consumption of the heart, and it actually shortened the life of the excised heart. Isolated frogs' hearts when perfused with a small amount of Ringer normally survived for 48 hours, but when 0·1 per cent. glucose was added the hearts regularly died in about 24 hours. The isolated heart of the frog, therefore, behaves like a diabetic tissue, but it does not appear to be benefited by insulin. It is of course possible that some essential factor is lacking, but this factor is not supplied by the addition of serum.

It is interesting to note that our results with the frog's heart accord with recent results obtained with minced skeletal muscle. Rothschild (19) found with this system that the addition of glucose did not raise the

oxygen consumption in the case of summer frogs, although it did raise the oxygen consumption in the case of winter frogs, and insulin was found to have no effect on the respiration either in the absence or presence of sugar.

Conclusions.

The study of the metabolism of the isolated heart of the frog shows that the power of this tissue to use carbohydrates is very limited. Even in the presence of serum, glucose, and insulin, the carbohydrate consumption only represents a small fraction of its total metabolism; moreover, the respiratory quotient usually is lower than 0·9.

The isolated heart loses small quantities of nitrogen, and if this nitrogen is the product of protein break-down, the quantity of nitrogen excreted is sufficient to account for about half the oxygen consumption.

Estimations of fat provide no evidence for consumption of fats either in the heart or in serum perfused through the heart. We conclude, therefore, that the isolated frog's heart uses proteins and, to a lesser extent, carbohydrates as sources of energy. This type of metabolism resembles the metabolism of amphibian embryos, which consume the following percentages: carbohydrates 7; proteins 71; fats 22 (Needham (20)).

Estimations of glycogen and the study of the R.Q. of the empty heart provide no evidence that the heart builds up glycogen from protein. During the first few hours of perfusion the heart loses more than two-thirds of its glycogen, but we have discovered no means whereby this loss can be prevented or glycogen storage be promoted. The results obtained with the frog's heart show a considerable resemblance to the results obtained with the dog's heart-lung preparation, but in the latter preparation carbohydrate consumption and glycogen storage are both increased when glucose and insulin are both present in excess, whereas we have hitherto failed to produce these effects in the frog's heart.

Experiments on the mammalian heart-lung, without addition of insulin, and on the isolated frog's heart agree in showing that such preparations use very little carbohydrate. A possible explanation is that such preparations lack some unknown factor essential for normal carbohydrate metabolism. The ease with which these preparations utilise non-carbohydrate sources of energy certainly accords with the hypothesis that the metabolism of carbohydrate is not the primary process that produces muscular contraction.

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